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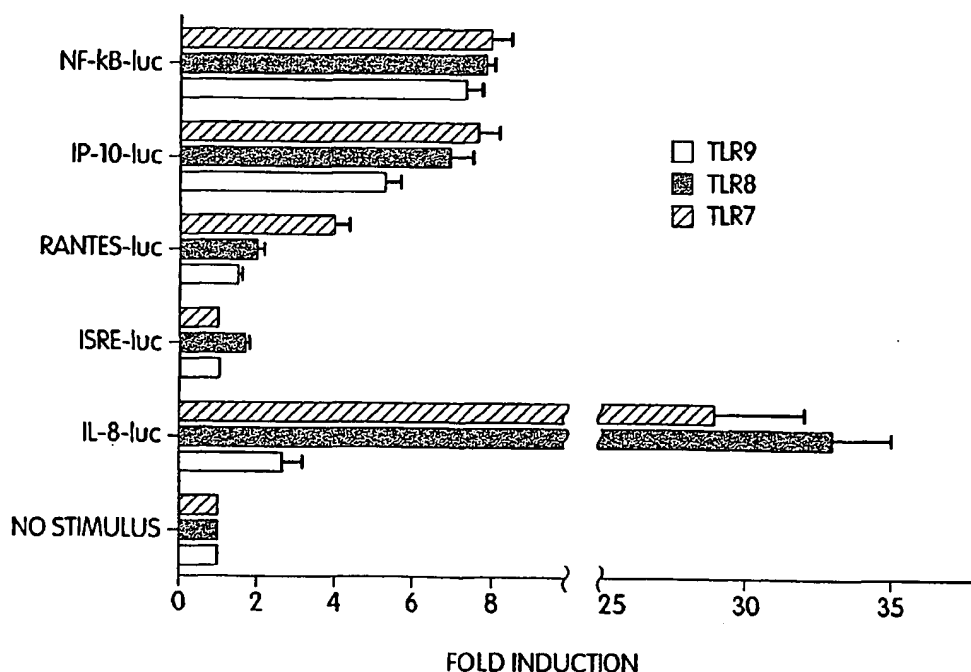
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(54) Title: TOLL-LIKE RECEPTOR 3 SIGNALING AGONISTS AND ANTAGONISTS



(57) Abstract: Compositions and methods are provided to identify, characterize, and optimize immunostimulatory compounds, their agonists and antagonists, working through TLR3.

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TOLL-LIKE RECEPTOR 3 SIGNALING AGONISTS AND ANTAGONISTS

Field of the Invention

The invention pertains to signal transduction by Toll-like receptor 3 (TLR3),
5 which is believed to be involved in innate immunity. More specifically, the invention
pertains to screening methods useful for the identification and characterization of TLR3
ligands, TLR3 signaling agonists, and TLR3 signaling antagonists.

Background of the Invention

10 Toll-like receptors (TLRs) are a family of at least ten highly conserved receptor
proteins (TLR1 – TLR10) which recognize pathogen-associated molecular patterns
(PAMPs) and act as key elements in innate immunity. As members of the pro-
inflammatory interleukin-1 receptor (IL-1R) family, TLRs share homologies in their
cytoplasmic domains called Toll/IL-1R homology (TIR) domains. PCT published
15 applications PCT/US98/08979 and PCT/US01/16766. Intracellular signaling
mechanisms mediated by TIRs appear generally similar, with MyD88 (Wesche H et al.
(1997) *Immunity* 7:837-47; Medzhitov R et al. (1998) *Mol Cell* 2:253-8; Adachi O et al.
(1998) *Immunity* 9:143-50; Kawai T et al. (1999) *Immunity* 11:115-22) and tumor
necrosis factor receptor-associated factor 6 (TRAF6; Cao Z et al. (1996) *Nature*
20 383:443-6; Lomaga MA et al. (1999) *Genes Dev* 13:1015-24) believed to have critical
roles. Signal transduction between MyD88 and TRAF6 is known to involve members
of the serine-threonine kinase IL-1 receptor-associated kinase (IRAK) family, including
at least IRAK-1 and IRAK-2. Muzio M et al. (1997) *Science* 278:1612-5.

Ligands for many but not all of the TLRs have been described. For instance, it
25 has been reported that TLR2 signals in response to peptidoglycan and lipopeptides.
Yoshimura A et al. (1999) *J Immunol* 163:1-5; Brightbill HD et al. (1999) *Science*
285:732-6; Aliprantis AO et al. (1999) *Science* 285:736-9; Takeuchi O et al. (1999)
Immunity 11:443-51; Underhill DM et al. (1999) *Nature* 401:811-5. TLR4 has been
reported to signal in response to lipopolysaccharide (LPS). Hoshino K et al. (1999) *J*
30 *Immunol* 162:3749-52; Poltorak A et al. (1998) *Science* 282:2085-8; Medzhitov R et al.
(1997) *Nature* 388:394-7. Bacterial flagellin has been reported to be a natural ligand
for TLR5. Hayashi F et al. (2001) *Nature* 410:1099-1103. TLR6, in conjunction with
with TLR2, has been reported to signal in response to proteoglycan. Ozinsky A et al.

(2000) *PNAS USA* 97:13766-71; Takeuchi O et al. (2001) *Int Immunol* 13:933-40.

Recently it was recently reported that TLR9 is a receptor for CpG DNA. Hemmi H et al. (2000) *Nature* 408:740-5.

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Summary of the Invention

The invention provides screening methods and compositions useful for the identification and characterization of compounds which themselves signal through Toll-like receptor 3 (TLR3) or which influence signaling through TLR3. Compounds which themselves signal through TLR3 are presumptively immunostimulatory.

10 Compounds which influence signaling through TLR3 include both agonists and antagonists of TLR3 signaling activity. The methods provided by the invention are adaptable to high throughput screening, thus accelerating the identification and characterization of previously unknown inducers, agonists, and antagonists of TLR3 signaling activity.

15 The methods of the invention rely at least in part on the ability to assess TLR3 signaling activity. It has surprisingly been discovered according to the present invention that reporter constructs having reporter genes under control of certain promoter response elements sensitive to TLR3 signaling activity are useful in the screening assays of the invention. For example it has been surprisingly discovered
20 according to the present invention that a reporter gene under control of interferon-specific response element (ISRE) is sensitive to TLR3 signaling activity.

It has also surprisingly been discovered according to the present invention that screening assays for TLR ligands and other assays involving TLR signaling activity can benefit from optimization for at least one of the variables of (a) concentration of test
25 and/or reference compound, (b) kinetics of the assay, and (c) selection of reporter. Interpretation of assay data can be influenced by each of these variables.

In one aspect the invention provides a screening method for identifying an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound under
30 conditions which, in absence of the test compound, permit a negative control response mediated by a TLR3 signal transduction pathway; (b) detecting a test response mediated by the TLR3 signal transduction pathway; and (c) determining the test

compound is an immunostimulatory compound when the test response exceeds the negative control response. In this and in all aspects of the invention, in one embodiment the screening method is performed on a plurality of test compounds. A test compound according to this and all aspects of the invention is in one embodiment a
5 member of a library of compounds, preferably a combinatorial library of compounds. Also in this and in all aspects of the invention, a test compound is preferably a small molecule, a nucleic acid, a polypeptide, an oligopeptide, or a lipid. In more preferred embodiments, the test compound is a small molecule or a nucleic acid. In one embodiment a test compound that is a nucleic acid is a CpG nucleic acid.

10 In another aspect the invention provides a screening method for identifying an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound under conditions which, in presence of a reference immunostimulatory compound, permit a reference response mediated by a TLR3 signal transduction pathway; (b) detecting a
15 test response mediated by the TLR3 signal transduction pathway; and (c) determining the test compound is an immunostimulatory compound when the test response equals or exceeds the reference response. In this and other aspects of the invention, a reference immunostimulatory compound is preferably a small molecule, a nucleic acid, a polypeptide, an oligopeptide, or a lipid. In one embodiment the reference
20 immunostimulatory compound is a CpG nucleic acid.

In a further aspect the invention provides a screening method for identifying a compound that modulates TLR3 signaling activity. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound and a reference immunostimulatory compound under conditions which,
25 in presence of the reference immunostimulatory compound alone, permit a reference response mediated by a TLR3 signal transduction pathway; (b) detecting a test-reference response mediated by the TLR3 signal transduction pathway; (c) determining the test compound is an agonist of TLR3 signaling activity when the test-reference response exceeds the reference response; and (d) determining the test compound is an
30 antagonist of TLR3 signaling activity when the reference response exceeds the test-reference response.

In yet another aspect the invention provides a screening method for identifying species specificity of an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) measuring a first species-specific response mediated by a TLR3 signal transduction pathway when a functional TLR3 of a first species is contacted with a test compound; (b) measuring a second species-specific response mediated by the TLR3 signal transduction pathway when a functional TLR3 of a second species is contacted with the test compound; and (c) comparing the first species-specific response with the second species-specific response. In a preferred embodiment the functional TLR3 of the first species is a human TLR3. In one preferred embodiment the functional TLR3 of the first species is a human TLR3 and the functional TLR3 of the second species is a mouse TLR3.

In preferred embodiments of the foregoing aspects of the invention, the response mediated by the TLR3 signal transduction pathway is measured quantitatively.

Also in preferred embodiments of the foregoing aspects of the invention, the functional TLR3 is expressed in a cell. For example, in one embodiment the cell is an isolated mammalian cell that naturally expresses the functional TLR3. Alternatively, in another embodiment the cell is an isolated mammalian cell that does not naturally express the functional TLR3, wherein the cell has an expression vector for TLR3. For example, in one preferred embodiment the cell is a human 293 fibroblast. In other embodiments, the functional TLR3 is part of a cell-free system.

Particularly useful in embodiments of the invention involving cells which express functional TLR3 are cells which include a reporter construct sensitive to TLR3 signaling. In one embodiment the cell includes an expression vector having an isolated nucleic acid which encodes a reporter construct selected from the group of nuclear factor-kappa B-luciferase (NF- κ B-luc), IFN-specific response element-luciferase (ISRE-luc), interleukin-6-luciferase (IL-6-luc), interleukin 8-luciferase (IL-8-luc), interleukin 12 p40 subunit-luciferase (IL-12 p40-luc), interleukin 12 p40 subunit-beta galactosidase (IL-12 p40- β -Gal), activator protein 1-luciferase (AP1-luc), interferon alpha-luciferase (IFN- α -luc), interferon beta-luciferase (IFN- β -luc), RANTES-luciferase (RANTES-luc), tumor necrosis factor-luciferase (TNF-luc), IP-10-luciferase

(IP-10-luc), and interferon-inducible T cell alpha chemoattractant-luciferase (I-TAC-luc). In a preferred embodiment the reporter construct is ISRE-luc.

In one embodiment according to each of the foregoing aspects of the invention, the functional TLR3 is part of a complex with a non-TLR protein selected from the group consisting of MyD88, IL-1 receptor associated kinase 1-3 (IRAK1, IRAK2, IRAK3), tumor necrosis factor receptor-associated factor 1-6 (TRAF1 - TRAF6), I κ B, NF- κ B, MyD88-adaptor-like (Mal), Toll-interleukin 1 receptor (TIR) domain-containing adapter protein (TIRAP), Tollip, Rac, and functional homologues and derivatives thereof. In a related embodiment functional TLR3 is part of a complex with a non-TLR protein listed above, excluding MyD88.

Also according to each of the foregoing aspects of the invention, in one embodiment the response mediated by a TLR3 signal transduction pathway is induction of a reporter gene under control of a promoter response element selected from the group consisting of ISRE, IL-6, IL-8, IL-12 p40, IFN- α , IFN- β , IFN- ω , RANTES, TNF, IP-10, and I-TAC. For example, in a preferred embodiment the reporter gene under control of a promoter response element is selected from the group consisting of ISRE-luc, IL-6-luc, IL-8-luc, IL-12 p40-luc, IL-12 p40- β -Gal, IFN- α -luc, IFN- β -luc, RANTES-luc, TNF-luc, IP-10-luc, and I-TAC-luc. In one preferred embodiment the reporter gene under control of a promoter response element is ISRE-luc. In yet another preferred embodiment the reporter gene is selected from the group consisting of IFN- α 1-luc and IFN- α 4-luc.

In yet another embodiment according to each of the foregoing aspects of the invention, the response mediated by a TLR3 signal transduction pathway is selected from the group consisting of (a) induction of a reporter gene under control of a minimal promoter responsive to a transcription factor selected from the group consisting of AP1, NF- κ B, ATF2, IRF3, and IRF7; (b) secretion of a chemokine; and (c) secretion of a cytokine. For example, in one preferred embodiment the response mediated by a TLR3 signal transduction pathway is induction of a reporter gene selected from the group consisting of AP1-luc and NF- κ B-luc. In another preferred embodiment the response mediated by a TLR3 signal transduction pathway is secretion of a type 1 IFN. In yet another preferred embodiment the response mediated by a TLR3 signal transduction

pathway is secretion of a chemokine selected from the group consisting of CCL5 (RANTES), CXCL9 (Mig), CXCL10 (IP-10), and CXCL11 (I-TAC).

The sensitivity and interpretation of the screening methods of the present invention can be optimized. Such optimization involves proper selection of any one or combination of (a) concentration of test and/or reference compound, (b) kinetics of the assay, and (c) reporter. Thus, further according to each of the first three aspects of the invention, in one embodiment the contacting a functional TLR3 with a test compound further entails, for each test compound, contacting with the test compound at each of a plurality of concentrations. For example, each test compound may be evaluated at various concentrations which differ by log increments. Also according to each of the foregoing aspects of the invention, in one embodiment the detecting is performed 4-12 hours, preferably 6-8 hours, following the contacting. Similarly, in yet another embodiment according to each of the foregoing aspects of the invention, the detecting is performed 16-24 hours following the contacting. Detecting performed 4-12 hours, preferably 6-8 hours, following the contacting is believed to be more sensitive to affinity of interaction than is detecting at later times. Detecting performed 16-24 hours or later following the contacting is believed to be more sensitive to stability and duration of receptor/ligand interaction. Furthermore, because certain reporter constructs are more sensitive to certain TLRs than others, proper matching of reporter to TLR assay is important to increase signal-to-noise ratio in the readout of a particular assay.

Brief Description of the Figures

This application includes examples which refer to figures or other drawings. It is to be understood that the referenced figures are illustrative only and are not essential to the enablement of the claimed invention.

Figure 1 is two paired bar graphs showing (A) the induction of NF- κ B and (B) the amount of IL-8 produced by 293 fibroblast cells transfected with human TLR9 in response to exposure to various stimuli, including CpG-ODN, GpC-ODN, LPS, and medium.

Figure 2 is a bar graph showing the induction of NF- κ B produced by 293 fibroblast cells transfected with murine TLR9 in response to exposure to various

stimuli, including CpG-ODN, methylated CpG-ODN (Me-CpG-ODN), GpC-ODN, LPS, and medium.

Figure 3 is a series of gel images depicting the results of reverse transcriptase-polymerase chain reaction (RT-PCR) assays for murine TLR9 (mTLR9), human TLR9 (hTLR9), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in untransfected control 293 cells, 293 cells transfected with mTLR9 (293-mTLR9), and 293 cells transfected with hTLR9 (293-hTLR9).

Figure 4 is a graph showing the degree of induction of NF- κ B-luc by various stimuli in stably transfected 293-hTLR9 cells.

Figure 5 is a graph showing the degree of induction of NF- κ B-luc by various stimuli in stably transfected 293-mTLR9 cells.

Figure 6 is a graph showing fold induction of response as a function of concentration for a series of four related immunostimulatory nucleic acids contacted with human 293 fibroblast cells stably transfected with murine TLR9 and NF- κ B-luc. Concentrations listed correspond to EC50 for each ligand.

Figure 7 is a graph showing kinetics of EC50 determinations for a series of five immunostimulatory nucleic acids contacted with human 293 fibroblast cells stably transfected with murine TLR9 and NF- κ B-luc.

Figure 8 is a graph showing kinetics of EC50 determinations for the same series of five immunostimulatory nucleic acids as in Figure 7 contacted with human 293 fibroblast cells stably transfected with human TLR9 and NF- κ B-luc.

Figure 9 is a graph showing kinetics of maximal activity (fold induction of response) for the same series of five immunostimulatory nucleic acids as in Figure 7 contacted with human 293 fibroblast cells stably transfected with murine TLR9 and NF- κ B-luc.

Figure 10 is a graph showing kinetics of maximal activity (fold induction of response) for the same series of five immunostimulatory nucleic acids as in Figure 7 contacted with human 293 fibroblast cells stably transfected with human TLR9 and NF- κ B-luc.

Figure 11 is a bar graph showing fold induction of response as measured using various luciferase reporter constructs (NF- κ B-luc, IP-10-luc, RANTES-luc, ISRE-luc,

and IL-8-luc) in combination with TLR7, TLR8, and TLR9, each TLR contacted with a specific reference TLR ligand.

Detailed Description of the Invention

5 The invention in certain aspects provides screening methods useful for the identification, characterization, and optimization of immunostimulatory compounds, including but not limited to immunostimulatory nucleic acids and immunostimulatory small molecules, as well as assays for the identification and optimization of agonists and antagonists of TLR3 signaling. The methods according to the invention include
10 both cell-based and cell-free assays. In certain preferred embodiments the screening methods are performed in a high throughput manner. The methods can be used to screen libraries of compounds for their ability to modulate immune activation that involves TLR3 signaling.

 In one aspect the invention provides a screening method for identifying an
15 immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound under conditions which, in absence of the test compound, permit a negative control response mediated by a TLR3 signal transduction pathway; (b) detecting a test response mediated by the TLR3 signal transduction pathway; and (c) determining the test
20 compound is an immunostimulatory compound when the test response exceeds the negative control response. In a second aspect the invention provides a screening method for identifying an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound under conditions which, in presence of a reference immunostimulatory
25 compound, permit a reference response mediated by a TLR3 signal transduction pathway; (b) detecting a test response mediated by the TLR3 signal transduction pathway; and (c) determining the test compound is an immunostimulatory compound when the test response equals or exceeds the reference response. It will be appreciated that these two aspects of the invention differ in that one involves comparison of the test
30 compound against a negative control and the other involves comparison of the test compound against a positive control.

For these and other aspects of the invention, the TLR3 is preferably a mammalian TLR3, such as human TLR3 or mouse TLR3. Nucleotide and amino acid sequences for human TLR3 and murine TLR3 have previously been described. The nucleotide sequence for human TLR3 cDNA can be found as GenBank accession no. NM_003265 (SEQ ID NO:1), and the deduced amino acid sequence for human TLR3, encompassing 904 amino acids, can be found as GenBank accession nos NP_003256 (SEQ ID NO:2). The nucleotide sequence for murine TLR3 cDNA can be found as GenBank accession no. AF355152 (SEQ ID NO:3), and the deduced amino acid sequence for murine TLR3, encompassing 905 amino acids, can be found as GenBank accession no. AAK26117 (SEQ ID NO:4).

As used herein, a "functional TLR3" shall refer to a polypeptide, including a full length naturally occurring TLR3 polypeptide as described above, which specifically binds a TLR3 ligand and signals via a Toll/interleukin-1 receptor (TIR) domain. In addition to full length naturally occurring TLR3, a functional TLR3 thus also refers to allelic variants, fusion proteins, and truncated versions of the same, provided the polypeptide specifically binds a TLR3 ligand and signals via a TIR domain. In a preferred embodiment, the functional TLR3 includes a human TLR3 extracellular domain having an amino acid sequence provided by amino acids 38-707 according to SEQ ID NO:2. In another preferred embodiment, the functional TLR3 includes a murine TLR3 extracellular domain having an amino acid sequence provided by amino acids 39-708 according to SEQ ID NO:4. Preferably, the functional TLR3 signals through a TIR domain of TLR3.

In certain embodiments of this and other aspects of the invention, the functional TLR3 is expressed, either naturally or artificially, in a cell. In some embodiments, a cell expressing TLR3 for use in the methods of the invention expresses TLR3 and no other TLR. Alternatively, in some embodiments a cell expressing TLR3 for use in the methods of the invention expresses both TLR3 and at least one other TLR, e.g., TLR7, TLR8, or TLR9. In one embodiment the cell is an isolated mammalian cell that naturally expresses functional TLR3. Cells and tissues known to express TLR3 include dendritic cells (DCs), intraepithelial cells, and placenta. Muzio M et al. (2000) *J Immunol* 164:5998-6004; Cario E et al. (2000) *Infect Immun* 68:7010-7; Rock FL et al. (1998) *Proc Natl Acad Sci USA* 95:588-93. The term "isolated" as used herein, with

reference to a cell or to a compound, means substantially free of or separated from components with which the cell or compound is normally associated in nature, e.g., other cells, nucleic acids, proteins, lipids, carbohydrates or *in vivo* systems to an extent practical and appropriate for its intended use.

5 In another embodiment the cell can be one that, as it occurs in nature, is not capable of expressing TLR3 but which is rendered capable of expressing TLR3 through the artificial introduction of an expression vector for TLR3. Examples of cell lines lacking TLR3 include, but are not limited to, human 293 fibroblasts (ATCC CRL-1573) and HEp-2 human epithelial cells (ATCC CCL-23). Examples of cell lines lacking
10 TLR9 include, but are not limited to, human 293 fibroblasts (ATCC CRL-1573), MonoMac-6, THP-1, U937, CHO, and any TLR9 knock-out. Typically the cell, whether it is capable of expressing TLR3 naturally or artificially, preferably has all the necessary elements for signal transduction initiated through the the TLR3 receptor. For example, it is believed that TLR9 signaling requires the adapter protein MyD88 in an
15 early step of signal transduction. In contrast, TLR3 appears not to require MyD88 but may require other factors further downstream, e.g., factors that induce mitogen-activated protein kinase (MAPK) and factors downstream of MAPK.

When indicated, introduction of a particular TLR into a cell or cell line is preferably accomplished by transient or stable transfection of the cell or cell line with a
20 TLR-encoding nucleic acid sequence operatively linked to a gene expression sequence (as described herein). For example, a cell artificially induced to express TLR3 for use in the methods of the invention includes a cell that has been transiently or stably transfected with a TLR3 expression vector. Any suitable method of transient or stable transfection can be employed for this purpose.

25 An expression vector for TLR3 will include at least a nucleotide sequence coding for a functional TLR3 polypeptide, operably linked to a gene expression sequence which can direct the expression of the TLR3 nucleic acid within a eukaryotic or prokaryotic cell. A "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which
30 facilitates the efficient transcription and translation of the nucleic acid to which it is operably linked. With respect to TLR3 nucleic acid, the "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer

combination, which facilitates the efficient transcription and translation of the TLR3 nucleic acid to which it is operably linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the

5 promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, β -actin promoter, and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the simian virus (e.g., SV40), papillomavirus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus (RSV),

10 cytomegalovirus (CMV), the long terminal repeats (LTR) of Moloney murine leukemia virus and other retroviruses, and the thymidine kinase (TK) promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent.

15 For example, the metallothionein (MT) promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

In general, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of

20 transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined TLR3 nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

25 Generally a nucleic acid coding sequence and a gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the transcription and/or translation of the nucleic acid coding sequence under the influence or control of the gene expression sequence. Thus the TLR3 nucleic acid sequence and the gene expression sequence are said to be "operably linked" when they

30 are covalently linked in such a way as to place the transcription and/or translation of the TLR3 coding sequence under the influence or control of the gene expression sequence. If it is desired that the TLR3 sequence be translated into a functional protein, two DNA

sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the TLR3 sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the TLR3 sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a TLR3 nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that TLR3 nucleic acid sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

In certain embodiments a TLR expression vector is constructed so as to permit tandem expression of two distinct TLRs, e.g., both TLR3 and a second TLR. Such a tandem expression vector can be used when it is desired to express two TLRs using a single transformation or transfection. Alternatively, a TLR3 expression vector can be used in conjunction with a second expression vector constructed so as to permit expression of a second TLR.

The screening assays can have any of a number of possible readout systems based upon a TLR/IL-1R signal transduction pathway. In preferred embodiments, the readout for the screening assay is based on the use of native genes or, alternatively, transfected or otherwise artificially introduced reporter gene constructs which are responsive to the TLR/IL-1R signal transduction pathway involving MyD88, TRAF, p38, and/or ERK. Häcker H et al. (1999) *EMBO J* 18:6973-82. These pathways activate kinases including κ B kinase complex and c-Jun N-terminal kinases. Thus reporter genes and reporter gene constructs particularly useful for the assays include, e.g., a reporter gene operatively linked to a promoter sensitive to NF- κ B. Examples of such promoters include, without limitation, those for NF- κ B, IL-1 β , IL-6, IL-8, IL-12 p40, CD80, CD86, and TNF- α . The reporter gene operatively linked to the TLR-sensitive promoter can include, without limitation, an enzyme (e.g., luciferase, alkaline phosphatase, β -galactosidase, chloramphenicol acetyltransferase (CAT), etc.), a bioluminescence marker (e.g., green-fluorescent protein (GFP, U.S. patent 5,491,084), etc.), a surface-expressed molecule (e.g., CD25), and a secreted molecule (e.g., IL-8, IL-12 p40, TNF- α). In certain preferred embodiments the reporter is selected from IL-

8, TNF- α , NF- κ B-luciferase (NF- κ B-luc; Häcker H et al. (1999) *EMBO J* 18:6973-82), IL-12 p40-luc (Murphy TL et al. (1995) *Mol Cell Biol* 15:5258-67), and TNF-luc (Häcker H et al. (1999) *EMBO J* 18:6973-82). In assays relying on enzyme activity readout, substrate can be supplied as part of the assay, and detection can involve
5 measurement of chemiluminescence, fluorescence, color development, incorporation of radioactive label, drug resistance, or other marker of enzyme activity. For assays relying on surface expression of a molecule, detection can be accomplished using flow cytometry (FACS) analysis or functional assays. Secreted molecules can be assayed using enzyme-linked immunosorbent assay (ELISA) or bioassays. These and other
10 suitable readout systems are well known in the art and are commercially available.

Thus a cell expressing a functional TLR3 and useful for the methods of the invention has, in some embodiments, an expression vector comprising an isolated nucleic acid which encodes a reporter construct useful for detecting TLR signaling. The expression vector comprising an isolated nucleic acid which encodes a reporter
15 construct useful for detecting TLR signaling can include a reporter gene under control of a minimal promoter responsive to a transcription factor believed by the applicant to be activated as a consequence of TLR3 signaling. Examples of such minimal promoters include, without limitation, promoters for the following genes: AP1, NF- κ B, ATF2, IRF3, and IRF7. In other embodiments the expression vector comprising an
20 isolated nucleic acid which encodes a reporter construct useful for detecting TLR signaling can include a gene under control of a promoter response element selected from IL-6, IL-8, IL-12 p40 subunit, a type 1 IFN, RANTES, TNF, IP-10, I-TAC, and ISRE. The promoter response element generally will be present in multiple copies, e.g., as tandem repeats. For example, an ISRE-luciferase reporter construct useful in
25 the invention is available from Stratagene (catalog no. 219092) and includes a 5x ISRE tandem repeat joined to a TATA box upstream of a luciferase reporter gene. As discussed further elsewhere herein, the reporter itself can be any gene product suitable for detection by methods recognized in the art. Such methods for detection can include, for example, measurement of spontaneous or stimulated light emission, enzyme
30 activity, expression of a soluble molecule, expression of a cell surface molecule, etc.

As mentioned above, the functional TLR3 is contacted with a test compound in order to identify an immunostimulatory compound. An immunostimulatory compound

is a natural or synthetic compound that is capable of inducing an immune response when contacted with an immune cell. In the context of the methods of the invention, an immunostimulatory compound refers to a natural or synthetic compound that is capable of inducing an immune response when contacted with an immune cell expressing a functional TLR3 polypeptide. Preferably the immune response is or involves activation of a TLR3 signal transduction pathway. Thus immunostimulatory compounds identified and characterized using the methods of the invention specifically include TLR3 ligands, i.e., compounds which selectively bind to TLR3 and induce a TLR3 signal transduction pathway. Immunostimulatory compounds in general include but are not limited to nucleic acids, including oligonucleotides and polynucleotides; oligopeptides; polypeptides; lipids, including lipopolysaccharides; carbohydrates, including oligosaccharides and polysaccharides; and small molecules. Accordingly, a "test compound" refers to nucleic acids, including oligonucleotides and polynucleotides; oligopeptides; polypeptides; lipids, including lipopolysaccharides; carbohydrates, including oligosaccharides and polysaccharides; and small molecules. Test compounds include compounds with known biological activity as well as compounds without known biological activity.

A "reference immunostimulatory compound" refers to an immunostimulatory compound that characteristically induces an immune response when contacted with an immune cell expressing a functional TLR polypeptide. In the screening methods of the invention, the reference immunostimulatory compound is a natural or synthetic compound that characteristically induces an immune response when contacted with an immune cell expressing a functional TLR3 polypeptide. Preferably the immune response is or involves activation of a TLR3 signal transduction pathway. Thus a reference immunostimulatory compound will characteristically induce a reference response mediated by a TLR3 signal transduction pathway when contacted with a functional TLR3 under suitable conditions. The reference response can be measured according to any of the methods described herein. Importantly, a reference immunostimulatory compound specifically includes a test compound identified as an immunostimulatory compound according to any one of the methods of the invention. Therefore a reference immunostimulatory compound can be a nucleic acid, including oligonucleotides and polynucleotides; an oligopeptide; a polypeptide; a lipid, including

lipopolysaccharides; a carbohydrate, including oligosaccharides and polysaccharides; or a small molecule.

Small molecules include naturally occurring, synthetic, and semisynthetic organic and organometallic compounds with molecular weight less than about 1.5 kDa.

5 Examples of small molecules include most drugs, subunits of polymeric materials, and analogs and derivatives thereof.

A "nucleic acid" as used herein with respect to test compounds and reference compounds used in the methods of the invention, shall refer to any polymer of two or more individual nucleoside or nucleotide units. Typically individual nucleoside or
10 nucleotide units will include any one or combination of deoxyribonucleosides, ribonucleosides, deoxyribonucleotides, and ribonucleotides. The individual nucleotide or nucleoside units of the nucleic acid can be naturally occurring or not naturally occurring. For example, the individual nucleotide units can include deoxyadenosine, deoxycytidine, deoxyguanosine, thymidine, and uracil. In addition to naturally
15 occurring 2'-deoxy and 2'-hydroxyl forms, individual nucleosides also include synthetic nucleosides having modified base moieties and/or modified sugar moieties, e.g., as described in Uhlmann E et al. (1990) *Chem Rev* 90:543-84. The linkages between individual nucleotide or nucleoside units can be naturally occurring or not naturally occurring. For example, the linkages can be phosphodiester, phosphorothioate,
20 phosphorodithioate, phosphoramidate, as well as peptide linkages and other covalent linkages, known in the art, suitable for joining adjacent nucleoside or nucleotide units. The nucleic acid test compounds and nucleic acid reference compounds typically range in size from 3-4 units to a few tens of units, e.g., 18-40 units.

The substituted purines and pyrimidines of the ISNAs include standard purines
25 and pyrimidines such as cytosine as well as base analogs such as C-5 propyne substituted bases. Wagner RW et al. (1996) *Nat Biotechnol* 14:840-4. Purines and pyrimidines include but are not limited to adenine, cytosine, guanine, thymine, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, and other naturally and non-naturally occurring nucleobases, substituted
30 and unsubstituted aromatic moieties.

Libraries of compounds that can be used as test compounds are available from various commercial suppliers, and they can be made to order using techniques well

known in the art, including combinatorial chemistry techniques. Especially in combination with high throughput screening methods, such methods including in particular automated multichannel methods of screening, large libraries of test compounds can be screened according to the methods of the invention. Large libraries
5 can include hundreds, thousands, tens of thousands, hundreds of thousands, and even millions of compounds.

Thus in preferred embodiments, the methods for screening test compounds can be performed on a large scale and with high throughput by incorporating, e.g., an array-based assay system and at least one automated or semi-automated step. For example,
10 the assays can be set up using multiple-well plates in which cells are dispensed in individual wells and reagents are added in a systematic manner using a multiwell delivery device suited to the geometry of the multiwell plate. Manual and robotic multiwell delivery devices suitable for use in a high throughput screening assay are well known by those skilled in the art. Each well or array element can be mapped in a
15 one-to-one manner to a particular test condition, such as the test compound. Readouts can also be performed in this multiwell array, preferably using a multiwell plate reader device or the like. Examples of such devices are well known in the art and are available through commercial sources. Sample and reagent handling can be automated to further enhance the throughput capacity of the screening assay, such that dozens,
20 hundreds, thousands, or even millions of parallel assays can be performed in a day or in a week. Fully robotic systems are known in the art for applications such as generation and analysis of combinatorial libraries of synthetic compounds. See, for example, U.S. patents 5,443,791 and 5,708,158.

A "CpG nucleic acid" or a "CpG immunostimulatory nucleic acid" as used
25 herein is a nucleic acid containing at least one unmethylated CpG dinucleotide (cytosine-guanine dinucleotide sequence, i.e. "CpG DNA" or DNA containing a 5' cytosine followed by 3' guanine and linked by a phosphate bond) and activates a component of the immune system. The entire CpG nucleic acid can be unmethylated or portions may be unmethylated but at least the C of the 5' CG 3' must be unmethylated.

30 In one embodiment a CpG nucleic acid is represented by at least the formula:



wherein X_1 and X_2 are nucleotides, N is any nucleotide, and N_1 and N_2 are nucleic acid sequences composed of from about 0-25 N's each. In some embodiments X_1 is adenine, guanine, or thymine and/or X_2 is cytosine, adenine, or thymine. In other embodiments X_1 is cytosine and/or X_2 is guanine.

- 5 Examples of CpG nucleic acids according to the invention include but are not limited to those listed in Table 1.

Table 1. Exemplary CpG Nucleic Acids

	AACGTTCT	
10	AAGCGAAAATGAAATTGACT	SEQ ID NO:39
	ACCATGGACGAACTGTTCCCTC	SEQ ID NO:40
	ACCATGGACGACCTGTTCCCTC	SEQ ID NO:41
	ACCATGGACGAGCTGTTCCCTC	SEQ ID NO:42
	ACCATGGACGATCTGTTCCCTC	SEQ ID NO:43
15	ACCATGGACGGTCTGTTCCCTC	SEQ ID NO:44
	ACCATGGACGTACTGTTCCCTC	SEQ ID NO:45
	ACCATGGACGTTCTGTTCCCTC	SEQ ID NO:46
	AGCGGGGGCGAGCGGGGCG	SEQ ID NO:47
	AGCTATGACGTTCCAAG	SEQ ID NO:48
20	ATCGACTCTCGAGCGTTCTC	SEQ ID NO:49
	ATGACGTTCTTGACGTT	SEQ ID NO:50
	ATGGAAGGTCCAACGTTCTC	SEQ ID NO:51
	ATGGAAGGTCCAGCGTTCTC	SEQ ID NO:52
	ATGGACTCTCCAGCGTTCTC	SEQ ID NO:53
25	ATGGAGGCTCCATCGTTCTC	SEQ ID NO:54
	CAACGTT	
	CACGTTGAGGGGCAT	SEQ ID NO:55
	CAGGCATAACGTTCCGTTAG	SEQ ID NO:56
	CCAACGTT	
30	CTGATTTCCTCCGAAATGATG	SEQ ID NO:57
	GAGAACGATGGACCTTCCAT	SEQ ID NO:58
	GAGAACGCTCCAGCACTGAT	SEQ ID NO:59
	GAGAACGCTCGACCTTCCAT	SEQ ID NO:60
	GAGAACGCTCGACCTTCGAT	SEQ ID NO:61
35	GAGAACGCTGGACCTTCCAT	SEQ ID NO:62
	GATTGCCTGACGTCAGAGAG	SEQ ID NO:63
	GCATGACGTTGAGCT	SEQ ID NO:64
	GCGGCGGGCGGCGCGGCCC	SEQ ID NO:65
	GCGTGCGTTGTCTGTTGTCGTT	SEQ ID NO:66
40	GCTAGACGTTAGCGT	SEQ ID NO:67
	GCTAGACGTTAGTGT	SEQ ID NO:68
	GCTAGATGTTAGCGT	SEQ ID NO:69
	GCTTGATGACTCAGCCGGAA	SEQ ID NO:70
	GGAATGACGTTCCCTGTG	SEQ ID NO:71

	GGGGTCAACGTTGACGGGG	SEQ ID NO:72
	GGGGTCAGTCTTGACGGGG	SEQ ID NO:73
	GTCCATTTCCCGTAAATCTT	SEQ ID NO:74
	GTCGCT	
5	GTCGTT	
	TACCGCGTGCGACCCCTCT	SEQ ID NO:75
	TCAACGTC	
	TCAACGTT	
	TCAGCGCT	
10	TCAGCGTGCGCC	SEQ ID NO:76
	TCATCGAT	
	TCCACGACGTTTTTCGACGTT	SEQ ID NO:77
	TCCATAACGTTCCCTGATGCT	SEQ ID NO:78
	TCCATAGCGTTCCTAGCGTT	SEQ ID NO:79
15	TCCATCACGTGCCTGATGCT	SEQ ID NO:80
	TCCATGACGGTCCTGATGCT	SEQ ID NO:81
	TCCATGACGTCCCTGATGCT	SEQ ID NO:82
	TCCATGACGTGCCTGATGCT	SEQ ID NO:83
	TCCATGACGTTCCCTGACGTT	SEQ ID NO:84
20	TCCATGACGTTCCCTGATGCT	SEQ ID NO:18
	TCCATGCCGGTCCTGATGCT	SEQ ID NO:85
	TCCATGCGTGCGTGCGTTTT	SEQ ID NO:86
	TCCATGCGTTGCGTTGCGTT	SEQ ID NO:87
	TCCATGGCGGTCCTGATGCT	SEQ ID NO:88
25	TCCATGTCGATCCTGATGCT	SEQ ID NO:89
	TCCATGTCGCTCCTGATGCT	SEQ ID NO:90
	TCCATGTCGGTCCTGATGCT	SEQ ID NO:91
	TCCATGTCGGTCCTGCTGAT	SEQ ID NO:92
	TCCATGTCGTCCCTGATGCT	SEQ ID NO:93
30	TCCATGTCGTTCCCTGATGCT	SEQ ID NO:94
	TCCATGTCGTTCCCTGTCGTT	SEQ ID NO:95
	TCCATGTCGTTTTTTGTCGTT	SEQ ID NO:96
	TCCTGACGTTCCCTGACGTT	SEQ ID NO:97
	TCCTGTCGTTCCCTGTCGTT	SEQ ID NO:98
35	TCCTGTCGTTCCCTGTCGTT	SEQ ID NO:99
	TCCTGTCGTTTTTTGTCGTT	SEQ ID NO:100
	TCCTGTCGTTCCCTGTCGTT	SEQ ID NO:101
	TCGATCGGGGCGGGCGGAGC	SEQ ID NO:102
	TCGTCGCTGTCTCCGCTTCTT	SEQ ID NO:103
40	TCGTCGCTGTCTCCGCTTCTTCTTGCC	SEQ ID NO:104
	TCGTCGCTGTCTGCCCTTCTT	SEQ ID NO:105
	TCGTCGCTGTTGTGCGTTTCTT	SEQ ID NO:106
	TCGTCGTCGTCGTT	SEQ ID NO:107
	TCGTCGTTGTGCGTTGTGCGTT	SEQ ID NO:108
45	TCGTCGTTGTGCGTTTTGTGCGTT	SEQ ID NO:109
	TCGTCGTTTTGTGCGTTTTGTGCGTT	SEQ ID NO:15
	TCTCCAGCGCGGCCAT	SEQ ID NO:110
	TCTCCAGCGGGCGCAT	SEQ ID NO:111

	TCTCCCAGCGTGC CG CCAT	SEQ ID NO:112
	TCTTCGAA	
	TGCAGATTGCGCAATCTGCA	SEQ ID NO:113
	TGTCGCT	
5	TGTCGTT	
	TGTCGTTGTCGTT	SEQ ID NO:114
	TGTCGTTGTCGTTGTCGTT	SEQ ID NO:115
	TGTCGTTGTCGTTGTCGTTGTCGTT	SEQ ID NO:116
	TGTCGTTTGTCTGTTTGTCTGTT	SEQ ID NO:117
10		

As used herein the term "response mediated by a TLR signal transduction pathway" refers to a response which is characteristic of an interaction between a TLR and an immunostimulatory compound that induces signaling events through the TLR. Such responses typically involve usual elements of Toll/IL-1R signaling, e.g., MyD88, TRAF, and IRAK molecules, although in the case of TLR3 the role of MyD88 is less clear than for other TLR family members. As demonstrated herein such responses include the induction of a gene under control of a specific promoter such as a NF- κ B promoter, increases in particular cytokine levels, increases in particular chemokine levels etc. The gene under the control of the NF- κ B promoter may be a gene which naturally includes an NF- κ B promoter or it may be a gene in a construct in which an NF- κ B promoter has been inserted. Genes which naturally include the NF- κ B promoter include but are not limited to IL-8, IL-12 p40, NF- κ B-luc, IL-12 p40-luc, and TNF-luc. Increases in cytokine levels may result from increased production or increased stability or increased secretion of the cytokines in response to the TLR-immunostimulatory compound interaction. Th1 cytokines include but are not limited to IL-2, IFN- γ , and IL-12. It has unexpectedly been discovered, according to the instant invention, that the promoter response element ISRE is directly activated as a result of signaling through the TLR3 signal transduction pathway, i.e., independent of IFN- γ production. Th2 cytokines include but are not limited to IL-4, IL-5, and IL-10. Chemokines of particular significance in the invention include but are not limited to CCL5 (RANTES), CXCL9 (Mig), CXCL10 (IP-10), and CXCL11 (I-TAC).

In another aspect the invention provides a screening method for identifying a compound that modulates TLR3 signaling activity. The method according to this aspect of the invention involves the steps of (a) contacting a functional TLR3 with a test compound and a reference immunostimulatory compound under conditions which,

in presence of the reference immunostimulatory compound alone, permit a reference response mediated by a TLR3 signal transduction pathway; (b) detecting a test-reference response mediated by the TLR3 signal transduction pathway; (c) determining the test compound is an agonist of TLR3 signaling activity when the test-reference
5 response exceeds the reference response; and (d) determining the test compound is an antagonist of TLR3 signaling activity when the reference response exceeds the test-reference response. A test-reference response refers to a type of test response as determined when a test compound and a reference immunostimulatory compound are simultaneously contacted with the TLR3. When a test compound is neither an agonist
10 nor an antagonist of TLR3 signaling activity, the test-reference response and the reference response are indistinguishable.

An agonist as used herein is a compound which causes an enhanced response of a TLR to a reference stimulus. The enhanced response can be additive or synergistic with respect to the response to the reference stimulus by itself. Furthermore, an agonist
15 can work directly or indirectly to cause the enhanced response. Thus an agonist of TLR3 signaling activity as used herein is a compound which causes an enhanced response of a TLR to a reference stimulus.

An antagonist as used herein is a compound which causes a diminished response of a TLR to a reference stimulus. Furthermore, an antagonist can work
20 directly or indirectly to cause the diminished response. Thus an antagonist of TLR3 signaling activity as used herein is a compound which causes a diminished response of a TLR to a reference stimulus.

In addition to identification and characterization of immunostimulatory compounds, agonists of TLR3 signaling, and antagonists of TLR3 signaling, the
25 methods of the invention also permit optimization of lead compounds. Optimization of a lead compound involves an iterative application of a screening method of the invention, further including the steps of selecting the best candidate at any given stage or round in the screening and then substituting it as a benchmark or reference in a subsequent round of screening. This latter process can further include selection of
30 parameters to modify in choosing and generating candidate test compounds to screen. For example, a lead compound from a particular round of screening can be used as a

basis to develop a focused library of new test compounds for use in a subsequent round of screening.

In another aspect the invention provides a screening method for identifying species specificity of an immunostimulatory compound. The method according to this aspect of the invention involves the steps of (a) measuring a first species-specific response mediated by a TLR3 signal transduction pathway when a functional TLR3 of a first species is contacted with a test compound; (b) measuring a second species-specific response mediated by the TLR3 signal transduction pathway when a functional TLR3 of a second species is contacted with the test compound; and (c) comparing the first species-specific response with the second species-specific response.

A species-specific TLR, including TLR3, is not limited to a human TLR, but rather can include a TLR derived from human or non-human sources. Examples of non-human sources include, but are not limited to, murine, rat, bovine, canine, feline, ovine, porcine, and equine. Other species include chicken and fish, e.g., aquaculture species.

The species-specific TLR, including TLR3, also is not limited to native TLR polypeptides. In certain embodiments the TLR can be, e.g., a chimeric TLR in which the extracellular domain and the cytoplasmic domain are derived from TLR polypeptides from different species. Such chimeric TLR polypeptides, as described above, can include, for example, a human TLR extracellular domain and a murine TLR cytoplasmic domain, each domain derived from the corresponding TLR of each species. In alternative embodiments, such chimeric TLR polypeptides can include chimeras created with different TLR splice variants or allotypes. Other chimeric TLR polypeptides useful for the screening methods of the invention include chimeric polypeptides created with a TLR of a first type, e.g., TLR3, and another TLR, e.g., TLR7, TLR8, or TLR9, of the same or another species as the TLR of the first type. Also contemplated are chimeric polypeptides which incorporate sequences derived from more than two polypeptides, e.g., an extracellular domain, a transmembrane domain, and a cytoplasmic domain all derived from different polypeptide sources, provided at least one such domain derives from a TLR3 polypeptide. As a further example, also contemplated are constructs such as include an extracellular domain of one TLR3, an intracellular domain of another TLR3, and a non-TLR reporter such as

luciferase, GFP, etc. Those of skill in the art will recognize how to design and generate DNA sequences coding for such chimeric TLR polypeptides.

It has also been discovered, according to the instant invention, that TLR-based screening assays, including but not limited to the TLR3-based assays described herein, are sensitive to parameters such as concentration of test compound, stability of test compound, kinetics of detection, and selection of reporter. These parameters can be optimized in order to derive the most information from a given screening assay. Importantly, the kinetics of detection appear to afford separation of types of information such as affinity of interaction and stability or duration of interaction. For example, measurements taken at earlier timepoints, e.g., after 6-8 hours of contact between TLR and test and/or reference compound, appear to reflect more information about affinity of interaction than do measurements obtained at later timepoints, e.g., after 16-24 or more hours of contact. In addition, while NF- κ B-driven reporters are generally useful in TLR-based screening assays like those of the instant invention, in some instances a reporter other than an NF- κ B-driven reporter will afford greater sensitivity. For example, the IL-8-luc reporter is significantly more sensitive to TLR7 and TLR8 than NF- κ B-luc. Selection of reporter thus appears to be TLR-dependent, while parameters relating to kinetics and concentration appear to be more compound-dependent. Thus in performing the screening methods of the instant invention, it is expected that the methods will be enhanced by inclusion of measurements obtained using at least two concentrations and two time points for each test compound. Typically at least three concentrations will be employed, spanning a two to three log-fold range of concentrations. Finer ranges of concentration can of course be employed under suitable circumstances, for instance based on results of an earlier screening performed using a wider initial range of concentrations.

The invention will be more fully understood by reference to the following examples. These examples, however, are merely intended to illustrate certain embodiments of the invention and are not to be construed to limit the scope of the invention.

Examples

Example 1. Expression Vectors for Human TLR3 (hTLR3) and Murine TLR3 (mTLR3)

To create an expression vector for human TLR3, human TLR3 cDNA was amplified by the polymerase chain method (PCR) from a cDNA made from human 293 cells using the primers

5'-GAAACTCGAGCCACCATGAGACAGACTTTGCCTTGTATCTAC-3' (sense, SEQ ID NO:9) and 5'-GAAAGAATTCTTAATGTACAGAGTTTTTGGATCCAAG-3' (antisense, SEQ ID NO:10). The primers introduce Xho I and EcoRI restriction endonuclease sites at their 5' ends for use in subsequent cloning into the expression

vector. The resulting amplification product fragment was cloned into pGEM-T Easy vector (Promega), isolated, cut with Xho I and EcoRI restriction endonucleases, ligated into an Xho I/EcoRI-digested pcDNA3.1 expression vector (Invitrogen). The insert was fully sequenced and translated into protein. The cDNA sequence corresponds to the published cDNA sequence for hTLR3, available as GenBank accession no. NM_003265 (SEQ ID NO:1). The open reading frame codes for a protein 904 amino acids long, having the sequence corresponding to GenBank accession no. NP_003256 (SEQ ID NO:2).

Table 2. cDNA Sequence for Human TLR3

(GenBank Accession No. NM_003265; SEQ ID NO:1)	
gcggccgcgt	cgacgaaatg tctggatttg gactaaagaa aaaaggaaag gctagcagtc 60
atccaacaga	atcatgagac agactttgcc ttgtatctac ttttgggggg gcctttttgcc 120
ctttgggatg	ctgtgtgcat cctccaccac caagtgcact gttagccatg aagttgctga 180
ctgcagccac	ctgaagttga ctcaggtagc cgatgatcta cccacaaaca taacagtgtt 240
25 gaaccttacc	cataatcaac tcagaagatt accagccgcc aacttcacaa ggtatagcca 300
gctaaactagc	ttggatgtag gatttaacac catctcaaaa ctggagccag aattgtgcca 360
gaaacttccc	atgttaaaag ttttgaacct ccagcacaat gagctatctc aactttctga 420
taaaaccttt	gccttctgca cgaatttgac tgaactccat ctcatgtcca actcaatcca 480
gaaaattaaa	aataatccct ttgtcaagca gaagaattta atcacattag atctgtctca 540
30 taatggcttg	tcatctacaa aattaggaac tcaggttcag ctggaaaatc tccaagagct 600
tctattatca	aacaataaaa ttcaagcgct aaaaagtga gaactggata tctttgcca 660
ttcatcttta	aaaaaattag agttgtcatc gaatcaaatt aaagagtttt ctccaggggtg 720
ttttcacgca	attggaagat tatttggcct ctttctgaac aatgtccagc tgggtcccag 780
ccttacagag	aagctatggt tggaaattagc aaacacaagc attcggaaatc tgtctctgag 840
35 taacagccag	ctgtccacca ccagcaatac aactttcttg ggactaaagt ggacaaatct 900
cactatgctc	gatctttcct acaacaactt aaatgtgggt ggtaacgatt cctttgcttg 960
gcttcacaa	ctagaatatt tcttcctaga gtataataat atacagcatt tgttttctca 1020
ctctttgcac	gggcttttca atgtgaggta cctgaatttg aaacgggtctt ttactaaaca 1080
aagtatttcc	cttgccctcac tccccaaagt tgatgatttt tcttttcagt ggctaaaatg 1140
40 tttggagcac	cttaacatgg aagataatga tattccaggc ataaaaagca atatgttcac 1200
aggattgata	aacctgaaat accttaagtct atccaactcc tttaacaagt tgccaacttt 1260
gacaaatgaa	acatttgtat cacttgctca ttctccctta cacatactca acctaaacca 1320
gaataaaatc	tcaaaaatag agagtgtatg tttctcttgg ttgggccacc tagaagtact 1380

	tgacctgggc	cttaatgaaa	ttgggcaaga	actcacaggc	caggaatgga	gaggtctaga	1440
	aaatatcttc	gaaatctatc	tttccataca	caagtacctg	cagctgacta	ggaactcctt	1500
	tgccctgggc	ccaagccttc	aacgactgat	gctccgaagg	gtggccctta	aaaatgtgga	1560
	tagctctcct	tcaccattcc	agcctcttcg	taacttgacc	attctggatc	taagcaacaa	1620
5	caacatagcc	aacataaatg	atgacatggt	ggagggtctt	gagaaactag	aaattctcga	1680
	tttgcagcat	aacaacttag	cacggctctg	gaaacacgca	aaccctgggt	gtcccattta	1740
	tttccataag	ggtctgtctc	acctccacat	ccttaacttg	gagtccaacg	gctttgacga	1800
	gatcccagtt	gaggtcttca	aggatttatt	tgaactaaag	atcatcgatt	taggattgaa	1860
	taatttaaac	acacttccag	catctgtctt	taataatcag	gtgtctctaa	agtcattgaa	1920
10	ccttcagaag	aattctcata	catccgttga	gaagaagggt	ttcgggccag	ctttcaggaa	1980
	cctgactgag	ttagatatgc	gctttaatcc	ctttgattgc	acgtgtgaaa	gtattgcctg	2040
	gtttgttaat	tggattaacg	agaccatac	caacatccct	gagctgtcaa	gccactacct	2100
	ttgcaacact	ccacctcact	atcatgggtt	cccagtgaag	ctttttgata	catcatcttg	2160
	caaagacagt	gccccctttg	aactcttttt	catgatcaat	accagtatcc	tggtgatttt	2220
15	tatctttatt	gtacttctca	tccactttga	gggctggagg	atatcttttt	attggaatgt	2280
	ttcagtagat	cgagtctctg	gtttcaaaag	aatagacaga	cagacagaac	agtttgaata	2340
	tgacagcatat	ataattcatg	cctataaaag	taaggattgg	gtctgggaac	atttctcttc	2400
	aattggaaaag	gaagaccaat	ctctcaaat	ttgtctggaa	gaaagggact	ttgaggcggg	2460
	tgtttttgaa	ctagaagcaa	ttgttaacag	catcaaaaag	agcagaaaaa	ttatttttgt	2520
20	tataacacac	catctattaa	aagaccatt	atgcaaaaag	ttcaaggtag	atcatgcagt	2580
	tcaacaagct	attgaacaaa	atctggattc	cattatattg	gttttccttg	aggagattcc	2640
	agattataaa	ctgaaccatg	cactctgttt	gcgaagagga	atgtttaaat	ctcactgcat	2700
	cttgaactgg	ccagttcaga	aagaacggat	aggtgccttt	cgtcataaat	tgcaagtagc	2760
	acttgatccc	aaaaactctg	tacattaaat	ttatttaaat	attcaattag	caaaggagaa	2820
25	actttctcaa	tttaaaaagt	tctatggcaa	atttaagttt	tccataaagg	tggtataatt	2880
	tgtttattca	tatttgtaaa	tgattatatt	ctatcacaat	tacatctctt	ctaggaaaat	2940
	gtgtctcctt	atttcaggcc	tatttttgac	aattgactta	attttacca	aaataaaaaca	3000
	tataagcacg	caaaaaaaaa	aaaaaaaaa				3029

30 Table 3. Amino Acid Sequence for Human TLR3

(GenBank Accession No. NP_003256; SEQ ID NO:2)

	MRQTLPCIYF	WGGLLPFGML	CASSTTKCTV	SHEVADCSHL	KLTQVPDDL	TNITVLNLTH	60
	NQLRRRLPAAN	FTRYSQLTSL	DVGFTNTISK	EPELCQKLPM	LKVLNLQHNE	LSQLSDKTFA	120
	FCTNLTELHL	MSNSIQKIKN	NPFVKQKNLI	TLDSLHNGLS	STKLGTQVQL	ENLQELLSN	180
35	NKIQAALKSEE	LDIFANSSLK	KLELSSNQIK	EFSPGCFHAI	GRLFGLFLNN	VQLGPSLTEK	240
	LCLELANTSI	RNLSLSNSQL	STTSNTTFLG	LKWTNLTMLD	LSYNNLNVVG	NDSFAWLPQL	300
	EYFFLEYNNI	QHLFSLHSLHG	LFNVRYLNLK	RSFTKQISISL	ASLPKIDDFS	FQWLKCLEHL	360
	NMEDNDIPGI	KSNMFTGLIN	LKYLSSLSNSF	TSRLTLTNET	FVSLAHSPLH	ILNLTKNKIS	420
	KIESDAFSLW	GHLEVLDLGL	NEIGQELTGQ	EWRGLENIFE	IYLSYNKYLO	LTRNSFALVP	480
40	SLQRLMLRRV	ALKNVDSPPS	PFQPLRNLT	LDLSNNNIAN	INDDMLEGLE	KLEILDLOHN	540
	NLARLWKHAN	PGGPYIFLKG	LSHLHILNLE	SNGFDEIPVE	VFKDLFELKI	IDLGLNNLNT	600
	LPASVFNNQV	SLKSLNLQKN	LITSVEKKVF	GPAFRNLTEL	DMRFNPFDC	CESIAWVFNW	660
	INETHNIPIE	LSSHLCNTP	PHYHGFPVRL	FDTSSCKDSA	PFELFFMINT	SILLIFIFIV	720
	LLIHFEWRI	SFYWNVSVHR	VLGFKEIDRQ	TEQFEYAAYI	IHAYKDKDWV	WEHFSSMEKE	780
45	DQSLKFCLEE	RDFEAGVFEL	EAIVNSIKRS	RKIIFVITHH	LLKDPLCKRF	KVHHAVQQAI	840
	EQNLDSIILV	FLEEIPDYKL	NHALCLRRGM	FKSHCILNWP	VQKERIGAFR	HKLQVALGSK	900
	NSVH						904

Corresponding nucleotide and amino acid sequences for murine TLR3

- 50 (mTLR3) are known. The nucleotide sequence of mTLR3 cDNA has been reported as GenBank accession no. AF355152, and the amino acid sequence of mTLR3 has been reported as GenBank accession no. AAK26117.

Table 4. cDNA Sequence for Murine TLR3

(GenBank Accession No. AF355152; SEQ ID NO:3)

	tagaatatga	tacagggatt	gcaccataa	tctgggctga	atcatgaaag	gggtgttcctc	60
5	ttatctaata	tactcctttg	ggggactttt	gtccctatgg	attcttcttg	tgtcttccac	120
	aaaccaatgc	actgtgagat	acaacgtagc	tgactgcagc	catttgaagc	taacacacat	180
	acctgatgat	cttccctcta	acataacagt	gttgaatctt	actcacaacc	aactcagaag	240
	attaccacct	accaacttta	caagatacag	ccaacttgct	atcttggatg	caggatttaa	300
	ctccatttca	aaactggagc	cagaactgtg	ccaaatactc	cctttgttga	aagtattgaa	360
10	cctgcaacat	aatgagctct	ctcagatttc	tgatcaaacc	tttgtcttct	gcacgaacct	420
	gacagaactc	gatctaattg	ctaactcaat	acacaaaatt	aaaagcaacc	ctttcaaaaa	480
	ccagaagaat	ctaatacaat	tagatttgct	tcataatgg	ttatcatcta	caaagttggg	540
	aacgggggtc	caactggaga	acctccaaga	actgctctta	gcaaaaaata	aaatccttgc	600
	gttgcggaagt	gaagaacttg	agtttcttgg	caattcttct	ttacgaaagt	tggacttgct	660
15	atcaaatcca	cttaaagagt	tctccccggg	gtgtttccag	acaattggca	agttattcgc	720
	cctcctcttg	aacaacgccc	aactgaaccc	ccacctcaca	gagaagcttt	gctgggaact	780
	ttcaaacaca	agcatccaga	atctctctct	ggctaacaac	cagctgctgg	ccaccagcga	840
	gagcactttc	ttggtgctga	agtggacaaa	tctcaccag	ctcgatcttt	cctacaacaa	900
	cctccatgat	gtcggcaacg	gttccttctc	ctatctccca	agcctgaggt	atctgtctct	960
20	ggagtacaac	aatatacagc	gtctgtcccc	tcgtcttttt	tatggactct	ccaacctgag	1020
	gtacctgagt	ttgaagcgag	catttactaa	gcaaagtgtt	tcacttgctt	cacatcccaa	1080
	cattgacgat	ttttcctttc	aatgggtaaa	atatttggaa	tatctcaaca	tggatgacaa	1140
	taatattcca	agtacaaaaa	gcaatacctt	cacgggattg	gtgagtctga	agtacctaa	1200
	tctttccaaa	actttcacaa	gtttgcaaac	tttaacaaat	gaaacatttg	tgctacttgc	1260
25	tcattctccc	ttgtctcact	tcaacttaac	gaaaaatcac	atctcaaaaa	tagcaaatgg	1320
	tactttctct	tgttagggcc	aactcaggat	acttgatctc	ggccttaatg	aaattgaact	1380
	aaaactcagc	ggccaggaat	ggagaggtct	gagaaatata	tttgagatct	acctatccta	1440
	taacaaatac	ctccaactgt	ctaccagttc	ctttgcattg	gtccccagcc	ttcaaagact	1500
	gatgctcagg	aggggtggcc	ttaaaaatgt	ggatatctcc	ccttcacctt	tccgccctct	1560
30	tcgtaacttg	accattcttg	acttaagcaa	caacaacata	gccaacataa	atgaggactt	1620
	gctggagggt	cttgagaatc	tagaaatcct	ggattttcag	cacaataact	tagccaggct	1680
	ctggaaacgc	gcaaaccocg	gtggtcccgt	taatttctct	aaggggctgt	ctcacctcca	1740
	catcttgaat	ttagagttca	acgggttaga	tgaaatccca	gtcggggttt	tcaaggaact	1800
	attcgaacta	aagagcatca	atctaggact	gaataactta	aacaaacttg	aaccattcat	1860
35	ttttgatgac	cagacatctc	taaggctcact	gaacctccag	aagaacctca	taacatctgt	1920
	tgagaaggat	gttttcgggc	cgccttttca	aaacctgaac	agtttagata	tgcgcttcaa	1980
	tccgttcgac	tgacagtggt	aaagtatttc	ctggtttgtt	aactggatca	accagaccca	2040
	cactaatatc	tttgagctgt	ccactcacta	cctctgtaac	actccacatc	attattatgg	2100
	cttccccctg	aagcttttct	atacatcatc	ctgtaaagac	agcgccccct	ttgaactcct	2160
40	cttcataatc	agcaccagta	tgctcctggg	ttttatactt	gtggtagtgc	tcattcacat	2220
	cgagggctgg	aggatctctt	tttactggaa	tgtttcagtg	catcggattc	ttgggttcaa	2280
	ggaaatagac	acacaggctg	agcagtttga	atatacagcc	tacataattc	atgcccataa	2340
	agacagagac	tgggtctggg	aacattttct	cccaatggaa	gaacaagacc	aatctctcaa	2400
	attttgctta	gaagaaaggg	actttgaagc	aggcgtcctt	ggacttgaag	caattgttaa	2460
45	tagcatcaaa	agaagccgaa	aaatcatttt	cgttatcaca	caccatttat	taaaagaccc	2520
	tctgtgcaga	agattcaagg	tacatcacgc	agttcagcaa	gctattgagc	aaaatctgga	2580
	ttcaattata	ctgatttttc	tccagaatat	tccagattat	aaactaaacc	atgcaactct	2640
	tttgcaaga	ggaatgttta	aatctcattg	catcttgaac	tggccagttc	agaaagaacg	2700
	gataaatgcc	tttcatcata	aattgcaagt	agcacttgga	tctcggaatt	cagcacatta	2760
50	aactcatttg	aagatttgga	gtcggtaaag	ggatagatcc	aatttataaa	ggtccatcat	2820
	gaatctaagt	tttacttgaa	agttttgtat	atatttttat	atgtatagat	gatgatatta	2880
	catcacaatc	caatctcagt	tttgaaatat	ttcggcttat	ttcattgaca	tctggtttat	2940
	tactccaaa	taaacacatg	ggcagttaaa	aacatcctct	attaatagat	taccatttaa	3000
	ttcttgaggt	gtatcacagc	tttaaagggt	tttaaatatt	tttatataaa	taagactgag	3060
55	agttttataa	atgtaatttt	ttaaactcga	agcttacttg	tgtagctcag	aaaggcctgg	3120
	aaattaatat	attagagagt	acttatttat	ctctgcctcc	ctctgtctcc	ctctgtctcc	3180
	agagtgttgc	ttttaagggt	atgtagcacc	acaccagct	atgtacgtgt	gggattttat	3240

aatgctcatt tttgagacgt ttatagaata aaagataatt gcttttatgg tataaggcta 3300
 cttgaggttaa 3310

Table 5. Amino Acid Sequence for Murine TLR3

5 (GenBank Accession No. AAK26117; SEQ ID NO:4)

	MKGCSSYLMY	SFGGLSLWI	LLVSSTNQCT	VRYNVADCSH	LKLTHIPDDL	PSNITVLNLT	60
	HNQLRRLPPT	NFTRYSQLAI	LDAGFNSISK	LEPELCQILP	LLKVLNLQHN	ELSQISDQTF	120
	VFCTNLTELD	LMSNSIHKIK	SNPFKNQKNL	IKLDLSHNGI	SSTKLGTGVQ	LENLQELLLA	180
	KNKILALRSE	ELEFLGNSSL	RKLDLSSNPL	KEFSPGCFQT	IGKLFALLLN	NAQLNPHLTE	240
10	KLCWELSNTS	IQNLSLANNQ	LLATSESTFS	GLKWTNLTQL	DLSYNNLHDV	GNGSFSYLP	300
	LRYLSLEYNN	IQRSPRSFY	GLSNLRYLSL	KRAFTKQSVS	LASHPNIDDF	SFQWLKYLEY	360
	LNMDNNIPS	TKSNTFTGLV	SLKYLSTLST	FTSLQTLTNE	TFVSLAHSPL	LTLNLTKNHI	420
	SKIANGTFSW	LGQLRILDLG	LNEIEQKLSG	QEWGRGLRNIF	EIYLSYNKYL	QLSTSSFALV	480
	PSLQRLMLRR	VALKNVDISP	SPFRPLRNLT	ILDLSNNNIA	NINEDLLEGL	ENLEILDFQH	540
15	NNLARLWKRA	NPGGPVNFLK	GLSHLHILNL	ESNGLDEIPV	GVFKNLFELK	SINLGLNNLN	600
	KLEPFIFDDQ	TSLRSLNLQK	NLITSVEKDV	FGPPFQNLNS	LDMRFNPFDC	TCEISISWVFN	660
	WINQTHTNIF	ELSTHYLCNT	PHHYGFPLK	LFDTSCKDS	APFELLFIIS	TSMLLVFILV	720
	VLLIHIEGWR	ISFYWNVSVH	RILGFKEIDT	QAEQFEYTAY	IIHAHKDRDW	VWEHFSPMEE	780
	QDQSLKFCLE	ERDFEAGVLG	LEAIVNSIKR	SRKIIFVITH	HLLKDPLCRR	FKVHHAVQQA	840
20	IEQNLDLSIIL	IFLQNPIDYK	LNHALCLRRG	MFKSHCILNW	PVQKERINAF	HHKLQVALGS	900
	RNSAH						905

Example 2. Method of Making IFN- α 4 Reporter Vector

A number of reporter vectors may be used in the practice of the invention.

- 25 Some of the reporter vectors are commercially available, e.g., the luciferase reporter vectors pNF- κ B-Luc (Stratagene) and pAP1-Luc (Stratagene). These two reporter vectors place the luciferase gene under control of an upstream (5') promoter region derived from genomic DNA for NF- κ B or AP1, respectively. Other reporter vectors can be constructed following standard methods using the desired promoter and a vector
- 30 containing a suitable reporter, such as luciferase, β -galactosidase (β -gal), chloramphenicol acetyltransferase (CAT), and other reporters known by those skilled in the art. Following are some examples of reporter vectors constructed for use in the present invention.

- IFN- α 4 is an immediate-early type 1 IFN. Sequence-specific PCR products for
- 35 the -620 to +50 promoter region of IFN- α 4 were derived from genomic DNA of human 293 cells and cloned into *Sma*I site of the pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -620 to +50 promoter region of IFN- α 4. The sequence of the -620 to +50 promoter region of IFN- α 4 is provided as SEQ ID NO:11 in Table 6.

Table 6. Nucleotide Sequence of the -620 to +50 Promoter Region of Human IFN- α 4
(SEQ ID NO:11)

	agaaaaat	tttaaaat	ttcattcata	tttttaggag	ttttgaatga	ttggatatgt	60
	aattatat	atattattaa	tgtgtatcta	tatatatttt	tattttgcat	atgtactttg	120
5	atacaaaat	tacatgaaca	aattacacta	aaagttattc	cacaaatata	cttatcaaat	180
	taagttaaat	gtcaatagct	tttaaaactta	aatttttagtt	taacttttct	gtcattcttt	240
	actttgaata	aaaagagcaa	actttgtagt	ttttatctgt	gaagtagagg	tatacgtaat	300
	atacataaat	agatatgcca	aatctgtgtt	attaaaat	catgaagatt	tcaattagaa	360
	aaaaatacca	taaaaggctt	tgagtgcagg	tgaaaaatag	gcaatgatga	aaaaaaatga	420
10	aaaacttttt	aaacacatgt	agagagtgcg	taaagaaagc	aaaaacagag	atagaaagta	480
	caactaggga	atttagaaaa	tggaaattag	tatgttcact	atttaagacc	tatgcacaga	540
	gcaaagtctt	cagaaaacct	agaggccgaa	gttcaagggt	atccatctca	agtagcctag	600
	caatatttgc	aacatcccaa	tggccctgtc	cttttcttta	ctgatggccg	tgctgggtgt	660
	cagctacaaa						670
15							

Example 3. Method of Making IFN- α 1 Reporter Vector

IFN- α 1 is a late type 1 IFN. Sequence-specific PCR products for the -140 to +9 promoter region of IFN- α 1 were derived from genomic DNA of human 293 cells and cloned into *Sma*I site of the pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -140 to +9 promoter region of IFN- α 1.

Example 4. Method of Making IFN- β Reporter Vector

IFN- β is an immediate-early type 1 IFN. The -280 to +20 promoter region of IFN- β was derived from the pUC β 26 vector (Algarte M et al. (1999) *J Virol* 73(4):2694-702) by restriction at *Eco*RI and *Taq*I sites. The 300 bp restriction fragment was filled in by Klenow enzyme and cloned into *Nhe*I-digested and filled in pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -280 to +20 promoter region of IFN- β . The sequence of the -280 to +20 promoter region of IFN- β is provided as SEQ ID NO:12 in Table 7.

Table 7. Nucleotide Sequence of the -280 to +20 Promoter Region of Human IFN- β
(SEQ ID NO:12)

	ttctcagg	gtttgtct	ctttgtct	tcccaagt	ctgtttta	caattgtt	tag	60
35	tcattcact	gaacttta	aaacattag	aaacctca	gtttgtaa	atctttcc	ct	120
	attatatata	tcataagata	ggagctta	aaagagtt	tagaaact	acataaat	gt	180
	atgacatagg	aaaactgaaa	gggagaagt	aaagtggg	aaatcctct	gataagag	ag	240
	gaccatctca	tataaaatag	ccataccccc	ggagaaag	gaattcta	actgcaac	ct	300

Example 5. Method of Making RANTES Reporter Vector

Transcription of the chemokine RANTES is believed to be regulated at least in part by IRF3 and by NF- κ B. Lin R et al. (1999) *J Mol Cell Biol* 19(2):959-66; Genin P et al. (2000) *J Immunol* 164:5352-61. A 483 bp sequence-specific PCR product

5 including the -397 to +5 promoter region of RANTES was derived from genomic DNA of human 293 cells, restricted with PstI and cloned into pCAT-Basic Vector (Promega) using HindIII (filled in with Klenow) and PstI sites (filled in). The -397 to +5 promoter region of RANTES was then isolated from the resulting RANTES/chloramphenicol acetyltransferase (CAT) reporter plasmid by restriction with

10 BglII and SalI, filled in with Klenow enzyme, and cloned into the NheI site (filled in with Klenow) of the pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -397 to +5 promoter region of RANTES. Comparison of the insert sequence -397 to +5 of Genin P et al. (2000) *J Immunol* 164:5352-61 and GenBank accession no. AB023652 (SEQ ID NO:13)

15 revealed two point deletions (at positions 105 and 273 of SEQ ID NO:13) which do not create new restriction sites. The sequence of the -397 to +5 promoter region of RANTES is provided as SEQ ID NO:14 in Table 8.

Table 8. Nucleotide Sequence of the -397 to +5 Promoter Region of Human RANTES**(SEQ ID NO:14)**

20	gatctgtaat	gaataagcag	gaactttgaa	gactcagtga	ctcagtgagt	aataaagact	60
	cagtgacttc	tgatcctgtc	ctaactgcc	ctccttggtg	tccaagaaa	gcggttcct	120
	gctctctgag	gaggaccct	tccctggaag	gtaaaactaa	ggatgtcagc	agagaaattt	180
	ttccaccatt	ggtgcttgg	caaagaggaa	actgatgagc	tactctaga	tgagagagca	240
25	gtgagggaga	gacagagact	cgaatttcg	gagctatttc	agttttcttt	tccgttttgt	300
	gcaatttcac	ttatgatacc	ggccaatgct	tggttgctat	tttggaact	ccccttaggg	360
	gatgccctc	aactggcct	ataaaggcc	agcctgagct	g		401

Table 9. Nucleotide Sequence of GenBank Accession No. AB023652 (SEQ ID NO:13)

30	agaaggcctt	acagtgagat	gggatcccag	tatttattga	gtttcctcat	tcataaaatg	60
	gggataataa	tagtaaatga	gttgacacgc	gctaagacag	tggaaatagtg	gctggcacag	120
	ataagccctc	ggtaaatggt	agccaataat	gatagagtat	gctgtaagat	atctttctct	180
	ccctctgctt	ctcaacaagt	ctctaataca	ttattccact	ttataaacia	ggaaatagaa	240
	ctcaaagaca	ttaagcactt	ttcccaaagg	tcgcttagca	agtaaattggg	agagacccta	300
35	tgaccaggat	gaaagcaaga	aattcccaca	agaggactca	ttccaactca	tatcttgtag	360
	aaagggtccc	aatgcccagc	tcagatcaac	tgcttcaatt	tacagtgtga	gtgtgctcac	420
	ctcctttggg	gactgtatat	ccagaggacc	ctcctcaata	aaacacttta	taaataacat	480
	ccttccatgg	atgagggaag	ggaggtaaga	tctgtaata	ataagcagga	actttgaaga	540
	ctcagtgact	cagtgaagta	taaagactca	gtgacttctg	atcctgtcct	aactgccact	600
40	ccttggtgtc	cccaagaaag	cggcttcctg	ctctctgagg	aggaccctt	ccctggaagg	660
	taaaactaag	gatgtcagca	gagaaatttt	tccaccattg	gtgcttggtc	aaagaggaaa	720

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ctgatgagct cactctagat gagagagcag tgaggagag acagagactc gaatttcg 780
aggctatttc agttttcttt tccgttttgt gcaatttcac ttatgatacc ggccaatgct 840
tggttgctat tttggaaact ccccttaggg gatgcccctc aactggccct ataaagggcc 900
agcctgagct gcagaggatt cctgcagagg atcaagacag cacgtggacc tcgcacagcc 960
5 tctcccacag gtaccatgaa ggtctccgcg gcagccctcg ctgtcatcct cattgctact 1020
gccctctgcg c 1031

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Example 6. Method of Making Human IL-12 p40 Reporter Vectors

Reporter constructs have been made using truncated (-250 to +30) and full length (-860 to +30) promoter regions derived from human IL-12 p40 genomic DNA. In one reporter construct the truncated IL-12 p40 promoter was cloned as a KpnI-XhoI insert into p β gal-Basic (Promega). The resulting expression vector includes a β gal gene under control of an upstream (5') -250 to +30 promoter region of human IL-12 p40. In a second reporter construct the full length IL-12 p40 promoter was cloned as a KpnI-XhoI insert into p β gal-Basic (Promega). The resulting expression vector includes a β gal gene under control of an upstream (5') -860 to +30 promoter region of human IL-12 p40. In a third reporter construct the truncated -250 to +30 promoter region of human IL-12 p40 was cloned into the pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -250 to +30 promoter region of human IL-12 p40. In a fourth reporter construct the full length IL-12 p40 promoter of human IL-12 p40 was cloned into the pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -860 to +30 promoter region of human IL-12 p40.

Example 7. Method of Making Human IL-6 Reporter Vectors

Reporter constructs are made using the -235 to +7 promoter region derived from human IL-6 genomic DNA. In one reporter construct the IL-6 promoter region is cloned as a KpnI-XhoI insert into pGL3-Basic Vector (Promega). The resulting expression vector includes a luciferase gene under control of an upstream (5') -235 to +7 promoter region derived from human IL-6 genomic DNA.

Example 8. Method of Making Human IL-8 Reporter Vectors

Reporter constructs have been made using a -546 to +44 and a truncated -133 to +44 promoter region derived from human IL-8 genomic DNA. Mukaida N et al. (1989) *J Immunol* 143:1366-71. In each reporter construct the IL-8 promoter region

was cloned as a KpnI-XhoI insert into pGL3-Basic Vector (Promega). One of the resulting expression vectors includes a luciferase gene under control of an upstream (5') -546 to +44 promoter region derived from human IL-8 genomic DNA. Another of the resulting expression vectors includes a luciferase gene under control of an upstream (5')
 5 -133 to +44 promoter region derived from human IL-8 genomic DNA.

Example 9. Sequence Comparison of Human TLR3 and Human TLR9

Human TLR3 and TLR9 are homologous proteins with several structural commonalities. Both appear to be transmembrane proteins with an extracellular
 10 domain and an intracellular domain. Common characteristics include a signal sequence and transmembranal domain. Similarities common to most TLRs include a cysteine rich domain and a TIR domain. Most TLRs have leucine rich repeats (LRR) in their extracellular domain. TLR3, TLR7, TLR8, and TLR9 appear to have similar structures. The regularity of the leucine repeats are shown below for TLR3 and TLR9.
 15 These four TLRs can be broken into two extracellular subdomains, domain 1 and 2, by virtue of a separation by an unstructured hinge region. TLR7, TLR8, and TLR9 have 14 LRR in domain 1 and 12 LRR in domain 2. TLR9 is a known nucleic acid binder, interacting with CpG-DNA. It has been suspected that TLR7 and TLR8 most likely also interact with nucleic acids. TLR3 has a similar 11 LRR in domain 1 and has 12
 20 LRR in domain 2, lacking the initial 3 repeats common to TLR7, TLR8, and TLR9. Based on structural consideration it is hypothesized that TLR3 interacts with nucleic acids or similar structures.

The structure of TLR3 differs from TLR7, TLR8, and TLR9 in an interesting character. Referring to Table 13, within the TIR domain it has been shown that a
 25 proline (shown in bold) is required for MyD88 interaction. MyD88 is required for TLR9 to transduce signal for the activation of NF- κ B. Both TLR7 and TLR8 also have this proline. TLR3 however has an alanine at this position (also shown in bold). It is believed by the applicant that this difference may disallow MyD88 interaction with TLR3 and thus result in an altered signal transduction pattern compared to, e.g., TLR9.

30

Table 10. Sequence Alignment of hTLR9 (SEQ ID NO:6) and hTLR3 (SEQ ID NO:2)

SIGNAL SEQUENCE

hTLR9	MGFCRSALHPLSLLVQAIMLAMTLALGTLPAFLPCELQPHGLVNCNW	47
hTLR3	MRQTLPCIYFWGGLLPFGMLCASSTTKCTVSHEVADC	37

DOMAIN 1 LEUCINE RICH REPEATS

5	hTLR9	LFLKSVPHFMSMAAPRGNVTSLSLSSN	73
	hTLR9	RIHHLHDSDFAHLP SLRHLNLKWN	97
10	hTLR9	CPPVGLSPMHFPCHMTIEPSTFLAVPTLEELNLSYN	133
	hTLR9	NIMTVPALPKSLISLSLSHT	153
15	hTLR3	SHLKLTVQVDDLPNTITVLNLTHN	61
	hTLR9	NILMLDSASLAGLHALRFLFMDGN	177
	hTLR3	QLRRLPAANFTRYSLTSLDVGFN	85
20	hTLR9	CYYKNPCRQALEVAPGALLGLGNLTHLSLKYN	209
	hTLR3	TISKLEPELCQKL PMLKVLNLQHN	109
	hTLR9	NLTVVPRNLPSSEYLLLSYN	230
	hTLR3	ELSQLSDKTFAFCTNLTELHLMSN	133
25	hTLR9	RIVKLAPEDLANLTALRVLDVGGN	254
	hTLR3	SIQIKNNPFVKQKNLITLDLSHN	157
	hTLR9	<u>CRRCDHAPNPCMECPRHFPQLHPDTFSHLSRLEGLVLKDS</u>	294
30	hTLR3	GLSSTKLGTQVQLENLQELLSSNN	181
	hTLR9	SLSWLNASWFRGLGNLRVLDLSEN	318
	hTLR3	KIQALKSEELDIFANSSLKKLELSSN	207
35	hTLR9	FLYKCITKTKAFQGLTQLRKLNLSFN	344
	hTLR3	QIKEFSPGCFHAIGRLFGLFLNNV	231
	hTLR9	YQKRVSFAHLSLAPSFGLVALKELDMHGI	374
	hTLR3	QLGPSLTEKLCLELANTSIRNLSLSNS	258
40	hTLR9	FFRSLDETTLRPLARLPMLQTLRLQMN	401
	hTLR3	QLSTTSNTTFLGLKWTNLMTLDLSYN	284
	hTLR9	FINQAQLGIFRAFPGLRYVDLSDN	425
45	hTLR3	NLNVVGND SFAWLPQLEYFFLEYN	308

HINGE REGION

	hTLR9	RISGASELTATMGEADGGEKVWLQPGDLAPAPV	458
50	hTLR3	NIQHLSHSLHGLFNRYLNLKRSFTKQISLA	341

DOMAIN 2 LEUCINE RICH REPEATS

	hTLR9	DTPSSEDFRPN CSTLNFTLDLSRN	482
	hTLR3	SLPKIDDFSQWLKCLEHLNMEDN	365
55	hTLR9	NLVTVPQEMFAQLSHLQCLRLSHN	506
	hTLR3	DIPGIKSNMFTGLINLKYSLSNS	389

	hTLR9	CISQAVNGSQFLPLTGLQVLDLSHN	531
	hTLR3	FTSLRTLNETFVSLAHSPLHILNLTKN	417
5	hTLR9	KLDLYHEHSFTELPRLEALDLSYN	555
	hTLR3	KISKIESDAFSWLGHLEVLDLGLN	441
	hTLR9	SQPFQMGGVGHNFVVAHLRTLRLSLAHN	585
10	hTLR3	EIGQELTGQEWGRLENIFEIYLSYN	466
	hTLR9	NIHSQVSQQLCSTSLRALDFSGN	608
	hTLR3	KYLQLTRNSFALVPSLQRLMLRRV	490
15	hTLR9	ALGHMWAEGDLYLHFFQGLSGLIWLDSLQN	638
	hTLR3	ALKNVDSSPSFPQPLRNLITLDSLNN	516
	hTLR9	RLHTLLPQTLRNLPKSLQVLRRLRDN	663
	hTLR3	NIANINDDMLEGLEKLEILDLQHN	540
20	hTLR9	YLAFFKWWSLHFLPKLEVLDLAGN	687
	hTLR3	NLARLWKHANPGGPYIFLKGLSHLHILNLESN	572
	hTLR9	QLKALTNGSLPAGTRLRLRDVSCN	711
25	hTLR3	GFDEIPVEVFKDLFELKIIDLGLN	596
	hTLR9	SISFVAPGFFSKAKELRELNLSAN	735
	hTLR3	NLNTLPASVFNNQVSLKSLNLQKN	620
30	hTLR9	ALKTVDHSWFGPLASALQILDVSAN	760
	hTLR3	LITSVEKKVFGPAFRNLTELDMRFN	645
CYSTEINE RICH DOMAIN			
35	hTLR9	PLHCACG**AAFMDFLLEVQAAPVGLPSRVKCGSPGQLQGLSIFAQD	805
	hTLR3	PFDCTCESIAWFVNWINETHTNIPELSSHYLCNTPPHYHGFPVRLFD	692
	hTLR9	LRLCLDEALSWDCA	820
	hTLR3	TSSCKDSAPFELFFM	707
TRANSMEMBRANAL DOMAIN			
40	hTLR9	LSLLAVALGLGVPMHLHL	838
	hTLR3	INTSILLIFIFIVLLIHF	725
TIR DOMAIN			
45	hTLR9	CGWDLWYCFHLCLAWLPWRGRQSGRDEDALPYDAFVVFDDKTQSAVAD	885
	hTLR3	EGWRISFYWNVSVHRVLGFKEIDRQTEQFE*YAAYIIHAYK***DKD	768
	hTLR9	WVYNELRGQLEECRGRWALRLCLEERDWLPGKTLFENLWASVYGSRK	932
	hTLR3	WVW***EHFSSMEKEDQSLKFCLEERDFEAGVFELEAIVNSIKRSRK	812
50	hTLR9	TLFVLAHTD*RVSGLLRASFLLAQQRLLEDKRDVVVLVILSPDGRRS	978
	hTLR3	IIFVITHLLKDPCKRFKVHAVQQAIEQNLDLSIILVFLEEIPDYK	859
	hTLR9	***RYVRLRQRLCRQSVLLWPHQPSGQRSFWAQLGMALTRDNHHFYN	1022
55	hTLR3	LNHALCLRRGMFKSHCILNWPVQKERIGAFRHKLQVALGSKNSVH	904
	hTLR9	RNFCQGPTAE	1032

Example 10. Reconstitution of TLR9 Signaling in 293 Fibroblasts

Methods for cloning murine and human TLR9 have been described in pending U.S. Patent Application No. 09/954,987 and corresponding published PCT application

5 PCT/US01/29229, both filed September 17, 2001, the contents of which are incorporated by reference. Human TLR9 cDNA and murine TLR9 cDNA in pT-Adv vector (from Clontech) were individually cloned into the expression vector pcDNA3.1(-) from Invitrogen using the EcoRI site. Utilizing a "gain of function" assay it was possible to reconstitute human TLR9 (hTLR9) and murine TLR9 (mTLR9)

10 signaling in CpG-DNA non-responsive human 293 fibroblasts (ATCC, CRL-1573). The expression vectors mentioned above were transfected into 293 fibroblast cells using the calcium phosphate method.

Table 11. cDNA Sequence for Human TLR9

15 (GenBank Accession No. AF245704; SEQ ID NO:5)

	aggctgggtat	aaaaatctta	cttcctctat	tctctgagcc	gctgctgccc	ctgtgggaag	60
	ggacctcgag	tgtgaagcat	ccttccctgt	agctgctgtc	cagtctgccc	gccagaccct	120
	ctggagaagc	ccctgcccc	cagcatgggt	ttctgccgca	gcgccctgca	cccgtgtct	180
	ctcctgggtgc	aggccatcat	gctggccatg	accctggccc	tgggtacctt	gcctgccttc	240
20	ctaccctgtg	agctccagcc	ccacggcctg	gtgaactgca	actggctgtt	cctgaagtct	300
	gtgccccact	tctccatggc	agcaccctgt	ggcaatgtca	ccagccttcc	cttgtcctcc	360
	aaccgcatcc	accacctcca	tgattctgac	tttggccacc	tgcccagcct	gcggcatctc	420
	aacctcaagt	ggaactgccc	gccggttgcc	ctcagcccca	tgcaacttccc	ctgccacatg	480
	accatcgagc	ccagcacctt	cttggctgtg	cccaccctgg	aagagctaaa	cctgagctac	540
25	aacaacatca	tgactgtgcc	tgcgctgccc	aaatccctca	tatccctgtc	cctcagccat	600
	accaacatcc	tgatgctaga	ctctgccagc	ctcgccggcc	tgcatgccct	gcgcttccta	660
	ttcatggagc	gcaactgtta	ttacaagaac	ccctgcaggc	aggcactgga	ggtggccccc	720
	ggtgcctccc	ttggcctggg	caacctcacc	cacctgtcac	tcaagtacaa	caacctcact	780
	gtggtgcccc	gcaacctgcc	ttccagcctg	gagtatctgc	tggtgtccta	caaccgcate	840
30	gtcaaaactgg	cgcctgagga	cctggccaat	ctgaccgccc	tgcgtgtgct	cgatgtgggc	900
	ggaaattgcc	gccgctgcga	ccacgctccc	aaccctgca	tggagtgcc	tcgtcacttc	960
	ccccagctac	atcccgatac	cttcagccac	ctgagccgtc	ttgaaggcct	ggtgttgaag	1020
	gacagtcttc	tctcctggct	gaatgccagt	tggttccgtg	ggctgggaaa	cctccgagtg	1080
	ctggacctga	gtgagaactt	cctctacaaa	tgcatcacta	aaaccaaggc	cttccagggc	1140
35	ctaacacagc	tgcgcaagct	taacctgtcc	ttcaattacc	aaaagagggt	gtcctttgcc	1200
	cacctgtctc	tgcccccttc	cttcgggagc	ctggctgccc	tgaaggagct	ggacatgcac	1260
	ggcactttct	tccgctcact	cgatgagacc	acgtcccggc	cactggcccc	cctgcccctg	1320
	ctccagactc	tgcgtctgca	gatgaacttc	atcaaccagg	cccagctcgg	catcttcagg	1380
	gccttccctg	gcctgcgcta	cgtggacctg	tccgacaacc	gcacagcgcc	agcttcggag	1440
40	ctgacagcca	ccatggggga	ggcagatgga	ggggagaagg	tctggctgca	gcctggggac	1500
	cttgctccgg	ccccagtgga	cactcccagc	tctgaagact	tcaggcccaa	ctgcagcacc	1560
	ctcaacttca	ccttgatctt	gtcacggaac	aacctggtga	ccgtgcagcc	ggagatgttt	1620
	gcccagctct	cgcacctgca	gtgcctgcgc	ctgagccaca	actgcatctc	gcaggcagtc	1680
	aatggctccc	agttcctgcc	gctgaccggg	ctgcaggtgc	tagacctgtc	ccgcaataag	1740
45	ctggacctct	accacgagca	ctcattcacg	gagctaccgc	gactggaggc	cctggacctc	1800
	agctacaaca	gccagccctt	tggcatgcag	ggcgtggggc	acaacttcag	cttcgtggct	1860
	cacctgcgca	ccctgcgcca	cctcagcctg	gcccacaaca	acatccacag	ccaagtgtcc	1920

	cagcagctct	gcagtacgtc	gctgcgggcc	ctggacttca	gcggcaatgc	actgggccat	1980
	atgtggggcg	agggagacct	ctatctgcac	ttcttccaag	gcctgagcgg	tttgatctgg	2040
	ctggacttgt	cccagaaccg	cctgcacacc	ctcctgcccc	aaaccctgcg	caacctcccc	2100
	aagagcctac	aggtgctgcg	tctccgtgac	aattacctgg	ccttctttaa	gtgggtggagc	2160
5	ctccacttcc	tgcccaaact	ggaagtccctc	gacctggcag	gaaaccggct	gaaggccctg	2220
	accaatggca	gcctgcctgc	tggcaccggg	ctccggaggg	tggatgtcag	ctgcaacagc	2280
	atcagcttcg	tggcccccg	cttcttttcc	aaggccaagg	agctgcgaga	gctcaacctt	2340
	agcgccaacg	ccctcaagac	agtggaccac	tcctggtttg	ggccccctggc	gagtgccctg	2400
	caaatactag	atgtaagcgc	caaccctctg	cactgcgcct	gtggggcggc	ctttatggac	2460
10	ttctctgtgg	aggtgcaggc	tgccgtgccc	ggctctgccc	gccgggtgaa	gtgtggcagt	2520
	ccggggccagc	tccagggcct	cagcatcttt	gcacaggacc	tgcgcctctg	cctggatgag	2580
	gccctctect	gggactgttt	cgccctctcg	ctgctggctg	tggctctggg	cctgggtgtg	2640
	cccatgctgc	atcacctctg	tggctgggac	ctctggtact	gcttccacct	gtgcctggcc	2700
	tggcttccct	ggcggggggc	gcaaagtggg	cgagatgagg	atgccctgcc	ctacgatgcc	2760
15	ttcgtggtct	tcgacaaaac	gcagagcgca	gtggcagact	gggtgtacaa	cgagcttcgg	2820
	gggcagctgg	aggagtgcg	tgggcgctgg	gcactccgcc	tgtgcctgga	ggaacgcgac	2880
	tggctgcctg	gcaaaaacct	ctttgagaac	ctgtgggcct	cggctctatg	cagccgcaag	2940
	acgctgtttg	tgctggccca	cacggaccgg	gtcagtggtc	tcttgccgcg	cagcttctctg	3000
	ctggcccagc	agcgctgct	ggaggaccgc	aaggacgtcg	tgggtgctgt	gatcctgagc	3060
20	cctgacggcc	gccgctcccg	ctacgtgcgg	ctgcgccagc	gcctctgccc	ccagagtgtc	3120
	ctcctctggc	cccaccagcc	cagtggtcag	cgcagcttct	gggcccagct	gggcatggcc	3180
	ctgaccaggg	acaaccacca	cttctataac	cggaaattct	gccagggacc	cacggccgaa	3240
	tagccgtgag	ccggaatcct	gcacggtgcc	acctccacac	tcacctcacc	tctgctgtcc	3300
	tggctcgacc	ctcccctgct	cgcctccctc	acccacacac	tgacacagag	ca	3352
25							

Table 12. Amino Acid Sequence for Human TLR9

(GenBank Accession No. AAF78037; SEQ ID NO:6)

	MGFCRSALHP	LSLLVQAIML	AMTLALGTLP	AFLPCELQPH	GLVNCNWLFL	KSVPHFSMAA	60
	PRGNVTSLSL	SSNRIHHLHD	SDFALPSPSR	HLNLKWNCP	VGLSPMHFPC	HMTIEPSTFL	120
30	AVPTLEELNL	SYNNIMTVPA	LPKSLISLSL	SHTNILMLDS	ASLAGLHALR	FLFMDGNCY	180
	KNPCRQALEV	APGALLGLGN	LTHLSLKYN	LTVVPRNLPS	SLEYLLLSYN	RIVKLAPEDL	240
	ANLTALRVLD	VGGNCRCDH	APNPCMECP	HFPQLHPDTF	SHLSRLEGLV	LKDSSLWLN	300
	ASWFRGLGNL	RVLDSLSENL	YKCITKTCAF	QGLTQLRKL	LSFNYQKRVS	FAHLSLAPSF	360
	GSLVALKELD	MHGIFRSLD	ETTLRPLARL	PMLQTLRLQ	NFINQAQLGI	FRAFPGLRYV	420
35	DLSDNRISGA	SELTATMGEA	DGGEKVLQ	GD LAPAPVD	PSEDFRPNC	STLNFTLDLS	480
	RNNLVTQPE	MFAQLSHLQ	LRLSHNCISQ	AVNGSQFLPL	TGLQVLDLSR	NKLDLYHEHS	540
	FTELPRLEAL	DLSYNSQPF	MQGVGHNF	VAHLRTLRL	SLAHNNIHSQ	VSQQLCSTSL	600
	RALDFSGNAL	GHWAEGLDY	LHFFQGLSG	IWLDSLQNR	HTLLPQTLRN	LPKSLQVLRL	660
	RDNYLAFFKW	WSLHFLPKLE	VLDLAGNRLK	ALTNGLPAG	TRLRLDVSC	NSISFVAPGF	720
40	FSKAKELREL	NLSANALKTV	DHSWFGPLAS	ALQILDVSAN	PLHCACGAAF	MDFLLEVQAA	780
	VPGLPSRVKC	GSPGQLQGLS	IFAQDLRLCL	DEALSWDCA	LSLLAVALGL	GVPMLHHLCG	840
	WDLWYCFHLC	LAWLPWRGRQ	SGRDEDALPY	DAFVVFDKTQ	SAVADWVYNE	LRGQLEECRG	900
	RWALRLCLEE	RDWLPKTLF	ENLWASVYGS	RKTLFVLAHT	DRVSGLLRAS	FLLAQQRLL	960
	DRKDVVVLVI	LSPDGRRSRY	VRLRQRLCRQ	SVLLWPHQPS	GQRSFWAQLG	MALTRDNHFF	1020
45	YNNFCQGPT	AE					1032

Table 13. cDNA Sequence for Murine TLR9

(GenBank Accession No. AF348140; SEQ ID NO:7)

	tgctcagaggg	agcctcgga	gaatcctcca	tctcccaaca	tggttctccg	tcgaaggact	60
50	ctgcaccct	tgctccctct	ggtacagget	gcagtgtctg	ctgagactct	ggccctgggt	120
	acctgcctg	ccttctacc	ctgtgagctg	aagcctcatg	gcctggtgga	ctgcaattgg	180
	ctgttctga	agtctgtacc	ccgtttctct	gcggcagcat	cctgctccaa	catcaccgcg	240
	ctctccttga	tctccaaccg	tatccaccac	ctgcacaact	ccgacttcgt	ccacctgtcc	300
	aacctgcggc	agctgaacct	caagtgaac	tgctccacca	ctggccttag	ccccctgcac	360

	ttctcttgcc	acatgaccat	tgagcccaga	accttctctgg	ctatgcgtac	actggaggag	420
	ctgaacctga	gctataatgg	tatcaccact	gtgccccgac	tgcccagctc	cctgggtgaat	480
	ctgagcctga	gccacaccaa	catcctgggt	ctagatgcta	acagcctcgc	cggcctatac	540
	agcctgcgcy	ttctcttcat	ggacgggaac	tgctactaca	agaacccctg	cacaggagcg	600
5	gtgaagggtga	ccccaggcgc	cctcctgggc	ctgagcaatc	tcacccatct	gtctctgaag	660
	tataacaacc	tcacaaaggt	gccccgccaa	ctgcccccca	gcctggagta	cctcctgggtg	720
	tcctataaacc	tcattgtcaa	gctggggcct	gaagacctgg	ccaatctgac	ctcccttcga	780
	gtacttgatg	tgggtgggaa	ttgccgtcgc	tgcgaccatg	cccccaatcc	ctgtatagaa	840
	tgtggccaaa	agtccctcca	cctgcaccct	gagaccttcc	atcacctgag	ccatctggaa	900
10	ggcctgggtgc	tgaaggacag	ctctctccat	acactgaact	cttctgggtt	ccaaggctctg	960
	gtcaacctct	cgggtgctgga	cctaagcgag	aactttctct	atgaaagcat	caaccacacc	1020
	aatgcctttc	agaacctaac	ccgcctgcgc	aagctcaacc	tgtccttcaa	ttaccgcaag	1080
	aaggatctct	ttgcccgcct	ccacctggca	agttccttca	agaacctggg	gtcactgcag	1140
	gagctgaaca	tgaacggcat	cttcttccgc	tcgctcaaca	agtacacgct	cagatggctg	1200
15	gccgatctgc	ccaaactcca	cactctgcat	cttcaaatga	acttcatcaa	ccaggcacag	1260
	ctcagcatct	ttggtacctt	ccgagccctt	cgctttgtgg	acttgtcaga	caatcgcatc	1320
	agtgggcctt	caacgctgtc	agaagccacc	cctgaagagg	cagatgatgc	agagcaggag	1380
	gagctgttgt	ctgcccgatcc	tcacccagct	ccactgagca	cccctgcttc	taagaacttc	1440
	atggacaggt	gtaagaactt	caagtccacc	atggacctgt	ctcggaaaca	cctgggtgact	1500
20	atcaagccag	agatgtttgt	caatctctca	cgctccaggt	gtcttagcct	gagccacaac	1560
	tccattgcac	aggtgtcaa	tggtctctag	ttcctgccgc	tgactaatct	gcaggtgctg	1620
	gacctgtccc	atacaaaact	ggacttgtac	cactggaaat	cgttcagtga	gctaccacag	1680
	ttgcaggccc	tggacctgag	ctacaacagc	cagcccttta	gcatgaaggg	tataggccac	1740
	aatttcagtt	ttgtggccca	tctgtccatg	ctacacagcc	ttagcctggc	acacaatgac	1800
25	attcataccc	gtgtgtcttc	acatctcaac	agcaactcag	tgaggtttct	tgacttcagc	1860
	ggcaacggta	tgggcccgc	gtgggatgag	gggggccttt	atctccattt	cttccaaggc	1920
	ctgagtggcc	tgctgaagct	ggacctgtct	caaaataaacc	tgcatatcct	ccggccccag	1980
	aaccttgaca	acctcccca	gagcctgaag	ctgctgagcc	tccgagacaa	ctacctatct	2040
	ttctttaact	ggaccagtct	gtccttctctg	cccaacctgg	aagtcctaga	cctggcaggc	2100
30	aaccagctaa	aggccctgac	caatggcacc	ctgcctaata	gcaccctcct	ccagaaactg	2160
	gatgtcagca	gcaacagtat	cgtctctgtg	gtcccagcct	tcttcgctct	ggcggtcgag	2220
	ctgaaagagg	tcaacctcag	ccacaacatt	ctcaagacgg	tggatcgctc	ctgggtttggg	2280
	cccatgtgta	tgaacctgac	agttctagac	gtgagaagca	accctctgca	ctgtgcctgt	2340
	ggggcagcct	tcgtagactt	actgttgagg	gtgcagacca	aggtgcctgg	cctggctaata	2400
35	gggtgtgaagt	gtggcagccc	cggccagctg	cagggccgta	gcatcttcgc	acaggacctg	2460
	cggctgtgcc	tggatgaggt	cctctcttgg	gactgctttg	gcctttcact	cttggctgtg	2520
	gccgtgggca	tgggtgtgcc	tatactgcac	catctctgcg	gctgggacgt	ctggtactgt	2580
	tttcatctgt	gctggcatg	gctacctttg	ctggcccgcg	gccgacgcag	cgcccaagct	2640
	ctccccatg	atgccttcgt	ggtgttcgat	aaggcacaga	gcgcagttgc	ggactgggtg	2700
40	tataacgagc	tgcgggtgcy	gctggaggag	cggcgcggtc	gccgagccct	acgcttgtgt	2760
	ctggaggacc	gagattggct	gcctggccag	acgctcttcg	agaacctctg	ggcttccatc	2820
	tatgggagcc	gcaagactct	atgtgtgctg	gcccacacgg	accgcgtcag	tggcctcctg	2880
	cgcaccagct	tcctgctggc	tcagcagcgc	ctggttgaag	accgcaagga	cgtggtgggtg	2940
	ttggtgatcc	tgcgtccgga	tgcccaccgc	tcccgtatg	tgcgactgcy	ccagcgtctc	3000
45	tgcgcccaga	gtgtgctctt	ctggccccag	cagcccaacg	ggcagggggg	cttctggggc	3060
	cagctgagta	cagccctgac	tagggacaac	cgccacttct	ataaccagaa	cttctgccgg	3120
	ggacctacag	cagaatagct	cagagcaaca	gctggaaaca	gctgcactct	catgcctggt	3180
	tcccagattg	ctctgectgc					3200

50 Table 14. Amino Acid Sequence for Murine TLR9

(GenBank Accession No. AAK29625; SEQ ID NO:8)

	MVLRRRTLHP	LSLLVQAVAL	AETLALGTLF	AFLPCELKPH	GLVDCNWLFL	KSVPRFSAAA	60
	SCSNITRLSL	ISNRIHHLHN	SDFVHLSNLR	QLNLKWNCP	TGLSPLHFSC	HMTIEPRFTL	120
	AMRTLEELNL	SYNGITVPR	LPSSLVNL	SHTNILVLDA	NSLAGLYSLR	VLFDGNCYY	180
55	KNPCTGAVKV	TPGALLGLSN	LTHLSLKYN	LTKVPRQLPP	SLEYLLVSYN	LIVKLGPEDL	240
	ANLTSRLVLD	VGGNCRCDH	APNPCIECGQ	KSLHLHPETF	HHLSHLEGLV	LKDSSLHTLN	300
	SSWFQGLVNL	SVLDLSENFL	YESINHNAF	QNLTRLRKLN	LSFNRYKKVS	FARLHLASSF	360

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KNLVSLQELN MNGIFFRSLN KYTLRWLADL PKLHTLHLQM NFINQAQLSI FGTFRALRFV 420
DLSDNRISGP STLSEATPEE ADDAEQEELL SADPHAPPLS TPASKNFMDR CKNFKFTMDL 480
SRNNLVTIKP EMFVNLSRLQ CLSLSHNSIA QAVNGSQFLP LTNLQVLDLS HNKLDLYHWK 540
SFSELPQLQA LDLSYNSQPF SMKGIGHNFS FVAHLSMLHS LSLAHNDIHT RVSSHLNSNS 600
5 VRFLDFSGNG MGRMWDEGGL YLHFFQGLSG LLKLDLSQNN LHILRPQNLD NLPKSLKLLS 660
LRDNYLSFFN WTSLSFLPNL EVLDLAGNQL KALTNGTLPN GTLLQKLDVS SNSIVSVVPA 720
FFALAVELKE VNLSHNILKT VDRSWFGPIV MNLTVLDVRS NPLHCACGAA FVDLLLEVQT 780
KVPGLANGVK CGSPGQLQGR SIFAQDLRLC LDEVLSWDCF GLSLLAVAVG MVVPILHHL 840
GWDVWYCFHL CLAWLPLLAR SRRSAQALPY DAFVVFDKAQ SAVADWVYNE LRVRLERERG 900
10 RRALRLCLED RDWLPGQTLF ENLWASIYGS RKTFLVLAHT DRVSGLLRTS FLLAQQRLL 960
DRKDVVVLVI LRPDAHRSRY VRLRQRLCRQ SVLFWPQQPN GGGFWAQLS TALTRDNRHF 1020
YNQNFCRGPT AE 1032

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Since NF- κ B activation is central to the IL-1/TLR signal transduction pathway
 15 (Medzhitov R et al. (1998) *Mol Cell* 2:253-258 (1998); Muzio M et al. (1998) *J Exp*
Med 187:2097-101), cells were transfected with hTLR9 or co-transfected with hTLR9
 and an NF- κ B-driven luciferase reporter construct. Human 293 fibroblast cells were
 transiently transfected with (Figure 1A) hTLR9 and a six-times NF- κ B-luciferase
 reporter plasmid (NF- κ B-luc, kindly provided by Patrick Baeuerle, Munich, Germany)
 20 or (Figure 1B) with hTLR9 alone. After stimulus with CpG-ODN (2006, 2 μ M,
 TCGTCGTTTGTCTGTTTGTCTGTT, SEQ ID NO:15), GpC-ODN (2006-GC, 2 μ M,
 TGCTGCTTTTGTGCTTTTGTGCTT, SEQ ID NO:16), LPS (100 ng/ml) or media,
 NF- κ B activation by luciferase readout (8h, Figure 1A) or IL-8 production by ELISA
 (48h, Figure 1B) were monitored. Results are representative of three independent
 25 experiments. Figure 1 shows that cells expressing hTLR9 responded to CpG-DNA but
 not to LPS.

Figure 2 demonstrates the same principle for the transfection of mTLR9.
 Human 293 fibroblast cells were transiently transfected with mTLR9 and the NF- κ B-
 luc construct (Figure 2). Similar data was obtained for IL-8 production (not shown).
 30 Thus expression of TLR9 (human or mouse) in 293 cells results in a gain of function
 for CpG-DNA stimulation similar to hTLR4 reconstitution of LPS responses.

To generate stable clones expressing human TLR9, murine TLR9, or either
 TLR9 with the NF- κ B-luc reporter plasmid, 293 cells were transfected in 10 cm plates
 (2 $\times 10^6$ cells/plate) with 16 μ g of DNA and selected with 0.7 mg/ml G418 (PAA
 35 Laboratories GmbH, Cölbe, Germany). Clones were tested for TLR9 expression by
 RT-PCR, for example as shown in Figure 3. The clones were also screened for IL-8

production or NF- κ B-luciferase activity after stimulation with ODN. Four different types of clones were generated.

293-hTLR9-luc:	expressing human TLR9 and 6-fold NF- κ B-luciferase reporter
5 293-mTLR9-luc:	expressing murine TLR9 and 6-fold NF- κ B-luciferase reporter
293-hTLR9:	expressing human TLR9
293-mTLR9:	expressing murine TLR9

Figure 4 demonstrates the responsiveness of a stable 293-hTLR9-luc clone after stimulation with CpG-ODN (2006, 2 μ M), GpC-ODN (2006-GC, 2 μ M), Me-CpG-ODN (2006 methylated, 2 μ M; TZGTZGTTTTGTZGTTTTGTZGTT, Z = 5-methylcytidine, SEQ ID NO:17), LPS (100 ng/ml) or media, as measured by monitoring NF- κ B activation. Similar results were obtained utilizing IL-8 production with the stable clone 293-hTLR9. 293-mTLR9-luc were also stimulated with CpG-ODN (1668, 2 μ M; TCCATGACGTTTCCTGATGCT, SEQ ID NO:18), GpC-ODN (1668-GC, 2 μ M; TCCATGAGCTTCCTGATGCT, SEQ ID NO:19), Me-CpG-ODN (1668 methylated, 2 μ M; TCCATGAZGTTTCCTGATGCT, Z = 5-methylcytidine, SEQ ID NO:20), LPS (100 ng/ml) or media, as measured by monitoring NF- κ B activation (**Figure 5**). Similar results were obtained utilizing IL-8 production with the stable clone 293-mTLR9. Results are representative of at least two independent experiments. These results demonstrate that CpG-DNA non-responsive cell lines can be stably genetically complemented with TLR9 to become responsive to CpG-DNA in a motif-specific manner. These cells can be used for screening of optimal ligands for innate immune responses driven by TLR9 in multiple species.

Example 11. Reconstitution of TLR3 Signaling in 293 Fibroblasts

Human TLR3 cDNA and murine TLR3 cDNA in pT-Adv vector (from Clontech) were individually cloned into the expression vector pcDNA3.1(-) from Invitrogen using the EcoRI site. The resulting expression vectors mentioned above were transfected into CpG-DNA non-responsive human 293 fibroblast cells (ATCC, CRL-1573) using the calcium phosphate method. Utilizing a "gain of function" assay it

was possible to reconstitute human TLR3 (hTLR3) and murine TLR3 (mTLR3) signaling in 293 fibroblast cells.

Since NF- κ B activation is central to the IL-1/TLR signal transduction pathway (Medzhitov R et al. (1998) *Mol Cell* 2:253-8; Muzio M et al. (1998) *J Exp Med* 5 187:2097-101), in a first set of experiments human 293 fibroblast cells were transfected with hTLR3 alone or co-transfected with hTLR3 and an NF- κ B-driven luciferase reporter construct.

Likewise, in a second set of experiments, 293 fibroblast cells were transfected with hTLR3 alone or co-transfected with hTLR3 and an IFN- α 4-driven luciferase 10 reporter construct (described in Example 2 above).

In a third group of experiments, 293 fibroblast cells were transfected with hTLR3 alone or co-transfected with hTLR3 and a RANTES-driven luciferase reporter construct (described in Example 5 above).

15 **Example 12. Proline to Histidine Mutation P915H in the TIR Domain of Human and MurineTLR9 Alters TLR9 Signaling**

Toll-like receptors have a cytoplasmic Toll/IL-1 receptor (TIR) homology domain which initiates signaling after binding of the adapter molecule MyD88. Medzhitov R et al. (1998) *Mol Cell* 2:253-8; Kopp EB et al. (1999) *Curr Opin Immunol* 20 11:15-8. Reports by others have shown that a single point mutation in the signaling TIR domain in murine TLR4 (Pro712 to His, P712H) or human TLR2 (Pro681 to His, P681H) abolishes host immune response to lipopolysaccharide or gram-positive bacteria, respectively. Poltorak A et al. (1998) *Science* 282:2085-8; Underhill DM et al. (1999) *Nature* 401:811-5. Through site-specific mutagenesis the equivalent proline 25 (P) at position 915 of human TLR9 and murine TLR9 were mutated to histidine (H; P915H). These mutations were generated by the use of the primers 5'-GCGACTGGCTGCATGGCAAACCCCTCTTTG-3' (SEQ ID NO:21) and 5'-CAAAGAGGGTTTTGCCATGCAGCCAGTCGC-3' (SEQ ID NO:22) for human TLR9 and the primers 5'-CGAGATTGGCTGCATGGCCAGACGCTCTTC-3' (SEQ 30 ID NO:23) and 5'-GAAGAGCGTCTGGCCATGCAGCCAATCTCG-3' (SEQ ID NO:24) for murine TLR9. Expression vectors for the mutant TLR9s, hTLR9-P915H

and mTLR9-P915H, were constructed and verified using standard recombinant DNA techniques.

For the stimulation of human TLR9 variant, hTLR9-P915H, 293 cells were transiently transfected with expression vector for hTLR9 or hTLR9-P915H and
5 stimulated after 16 hours with ODN 2006 or ODN 1668 at various concentrations. Likewise for the stimulation of murine TLR9 variant, mTLR9-P915H, 293 cells were transiently transfected with expression vector for mTLR9 or mTLR9-P915H and stimulated after 16 hours with ODN 2006 or ODN 1668 at various concentrations. After 48 hours of stimulation, supernatant was harvested and IL-8 production was
10 measured by ELISA. Results demonstrated that TLR9 activity can be destroyed by the P915H mutation in the TIR domain of both human and murine TLR9.

Example 13. Exchange of the TIR Domain Between Human TLR3 and Human TLR9 (hTLR3-TIR9 and hTLR9-TIR3)

15 While TLR3 and TLR9 share many structural features, TLR3, by virtue of its having an alanine rather than proline at a critical position in the TIR domain, may not be able to signal via MyD88 as does TLR9. The chimeric TLRs described here can be used in the screening assays of the invention. To generate molecules consisting of human extracellular TLR3 and the TIR domain of human TLR9 (hTLR3-TIR9), the
20 following approach can be used. Through site-specific mutagenesis a ClaI restriction site is introduced in human TLR3 and human TLR9. For human TLR9 the DNA sequence 5'-GGCCTCAGCATCTTT-3' (3026-3040, SEQ ID NO:25) is mutated to 5'-GGCCTATCGATTTTT-3' (SEQ ID NO:26), introducing a ClaI site (underlined in the sequence) but leaving the amino acid sequence (GLSIF, aa 798-802) unchanged. For
25 human TLR3 the DNA sequence 5'-GGGTTCCAGTGAGA-3' (2112-2126, SEQ ID NO:27) is mutated to 5'-GGGTTATCGATTAGA-3' (SEQ ID NO:28), introducing a ClaI site and creating the amino acid sequence (GLSIR, aa 685-689) which differs in three positions (aa 686, 687, 688) from the wildtype human TLR3 sequence (GFPVR, aa 685-689).

30 **hTLR3-TIR9.** The primers used for human TLR9 are 5'-CAGCTCCAGGGCCTATCGATTTTGCACAGGACC-3' (SEQ ID NO:29) and 5'-GGTCCTGTGCAAAAATCGATAGGCCCTGGAGCTG-3' (SEQ ID NO:30). For

creating an expression vector containing the extracellular portion of human TLR3 connected to the TIR domain of human TLR9, the human TLR3 expression vector is cut with ClaI and limiting amounts of EcoRI and the fragment coding for the TIR domain of human TLR9 generated by a ClaI and EcoRI digestion of human TLR9 expression vector is ligated in the vector fragment containing the extracellular portion of hTLR3. Transfection into *E.coli* yields the expression vector hTLR3-TIR9 (human extracellular TLR3-human TLR9 TIR domain). The expressed product of hTLR3-TIR9 can interact with TLR3 ligands and also signal through an MyD88-mediated signal transduction pathway.

10 **hTLR9-TIR3.** A fusion construct with the extracellular domain of hTLR9 and the TIR domain of hTLR3 is prepared using an analogous strategy. For creating an expression vector containing the extracellular portion of human TLR9 connected to the TIR domain of human TLR3, the human TLR9 expression vector is cut with ClaI and limiting amounts of EcoRI and the fragment coding for the TIR domain of human TLR3 generated by a ClaI and EcoRI digestion of human TLR3 expression vector is ligated in the vector fragment containing the extracellular portion of hTLR9. Transfection into *E.coli* yields the expression vector hTLR9-TIR3 (human extracellular TLR9-human TLR3 TIR domain). The expressed product of hTLR9-TIR3 can interact with TLR9 ligands, e.g., CpG DNA, and signal through a signal transduction pathway in a manner like TLR3.

Example 14. Sensitive in vitro Assay for Detecting Ligand Affinity Differences for a TLR

Human 293 fibroblast cells stably transfected with murine TLR9 and an NF- κ B-luciferase reporter were stimulated for 16 hours with the following fully phosphorothioated oligodeoxynucleotides (ODN):

5890: T*C*C*A*T*G*A*C*G*T*T*T*T*G*A*T*G*T*T (SEQ ID NO:31)
 5895: T*C*C*A*T*G*A*C*G*T*T*T*T*G*A*T*G (SEQ ID NO:32)
 30 5896: T*C*C*A*T*G*A*C*G*T*T*T*T*G*A (SEQ ID NO:33)
 5897: T*C*C*A*T*G*A*C*G*T*T*T*T (SEQ ID NO:34)

Concentration of the stimulus was titrated between 10 μ M and 2 nM. The data is plotted in Figure 6 as fold induction of NF- κ B luciferase, relative to unstimulated

compound, versus ODN concentration. The data displays typical first-order binding curves from which EC50 or maximal activity can be determined. EC50 is defined as the concentration of the ligand stimulus that results in 50% maximal activation. As shown in the figure, the EC50 ranges from 42 nM for ODN 5890 to 1220 nM for ODN 5897. The assay demonstrates sensitive differentiation between subtle changes in ligand.

Example 15. Influence of Assay Kinetics on TLR Screening Assays

Curves were prepared as in the previous Example 14 with the following ODN strands, where * indicates phosphorothioate and _ indicates phosphodiester linkage:

ODN 5890: TGC*CA*TG*AC*G*TT*TT*G*AT*G*TT	(SEQ ID NO:35)
ODN 5891: TCG*TC*G*TT*TT*G*TC*G*TT*TT*G*TC*G*TT	(SEQ ID NO:36)
ODN 5892: TCG*TC*G*TT*TT*G*TC*G*TT*TT*G*TC*G*TT	(SEQ ID NO:37)
ODN 5893: TCG*TC*G*TT*TT*G*TC*G*TT*TT*G*TC*G*TT	(SEQ ID NO:15)
ODN 5894: TCG*CA*TG*AC*G*TT*TT*G*AT*G*TT	(SEQ ID NO:38)

A family of stimulation curves was determined at various times of assay incubation between 1 and 24 hours. The EC50 was determined for each ligand at each time point. The EC50 was then plotted versus time to yield the resultant curves shown in Figure 7.

As evident from Figure 7, it is demonstrated that the kinetics of activation vary dependent on the ligand tested. Because luciferase has a three-hour half-life, the signal is transient and requires constant promoter-driven activation to be maintained. The response is directly related to the signal delivered by the ligand/receptor complex. This analysis of time kinetics in such a fashion allows one to determine both affinity of ligand/receptor interaction and the availability of the ligand to the receptor through time. The principle is demonstrated as follows. The ODN 5890 is of higher affinity compared to the ODN 2006. When the ligand is made more labile to destruction by incorporating less stable diester linkages, the activity curves turn upward with time such as for ODN 5746, 5902 and 5497.

In the context of a screening assay for TLR/ligand interactions, limiting the assay to one time point would bias the assay. At 24 hours it would appear that only ODN 2006 and 5890 were ligand candidates, however this is clearly not the case. The assay also demonstrates that earlier time points, such as 6 hours in this example, would be the optimal time point for determining the greatest difference between

receptor/ligand affinities. Thus optimization of the screening assay can be adjusted depending on the desired information to be obtained from the screen, e.g., higher affinity of interaction versus stability and duration of receptor/ligand interaction.

Figure 8 demonstrates the same principles shown with a murine TLR as in this example can be applied independent of the TLR utilized. For this set of data a 293 cell stably transfected with human TLR9 and NF- κ B-luciferase was used.

Example 16. Influence of Assay Kinetics on Maximal Activities in TLR Screening Assays

Data was collected as in the previous Example 15, however the maximal activity (maximal fold induction) was plotted versus time in Figures 9 and 10. Such data analysis results in a prediction of biological efficacy. As can be seen from these figures, the lower affinity ODN, e.g., ODN 2006 and 5890 as demonstrated by the EC50 curves of Example 15, are clearly less efficient at delivering high activity.

Example 17. Differential Outcomes of TLR Screening Assays Dependent on Promoter Utilization

Human 293 fibroblast cells were transiently transfected with expression vector for TLR 7, TLR8, or TLR9 and one of the following reporter constructs bearing the following promoters driving the luciferase gene: NF- κ B-luc, IP-10-luc, RANTES-luc, ISRE-luc, and IL-8-luc. The cells were stimulated for 16h with the maximal activity concentration of specific ligand. TLR9 was stimulated with CpG ODN 2006; TLR8 and TLR7 were stimulated with the imidazolquinalone R848. Results are shown in Figure 11. As evident from the figure, the promoter used influences the outcome of the screening assay dependent on the TLR in question. For example, NF- κ B is a reliable marker for all TLRs tested, whereas in this set of experiments ISRE was only functional to some extent for TLR8. The IL-8 promoter is particularly sensitive for TLR7 or TLR8 screening assays but would be much less efficient in TLR9 assays.

What is claimed is:

Claims

1. A screening method for identifying an immunostimulatory compound,
comprising:
5 contacting a functional TLR3 with a test compound under conditions which, in
absence of the test compound, permit a negative control response mediated by a
TLR3 signal transduction pathway;
 detecting a test response mediated by the TLR3 signal transduction pathway;
and
10 determining the test compound is an immunostimulatory compound when the
test response exceeds the negative control response.
2. A screening method for identifying an immunostimulatory compound,
comprising:
15 contacting a functional TLR3 with a test compound under conditions which, in
presence of a reference immunostimulatory compound, permit a reference response
mediated by a TLR3 signal transduction pathway;
 detecting a test response mediated by the TLR3 signal transduction pathway;
and
20 determining the test compound is an immunostimulatory compound when the
test response equals or exceeds the reference response.
3. A screening method for identifying a compound that modulates TLR3 signaling
activity, comprising:
25 contacting a functional TLR3 with a test compound and a reference
immunostimulatory compound under conditions which, in presence of the reference
immunostimulatory compound alone, permit a reference response mediated by a
TLR3 signal transduction pathway;
 detecting a test-reference response mediated by the TLR3 signal transduction
30 pathway;
 determining the test compound is an agonist of TLR3 signaling activity when
the test-reference response exceeds the reference response; and

determining the test compound is an antagonist of TLR3 signaling activity when the reference response exceeds the test-reference response.

4. A screening method for identifying species specificity of an immunostimulatory
5 compound, comprising:
 measuring a first species-specific response mediated by a TLR3 signal
transduction pathway when a functional TLR3 of a first species is contacted with a
test compound;
 measuring a second species-specific response mediated by the TLR3 signal
10 transduction pathway when a functional TLR3 of a second species is contacted with
the test compound; and
 comparing the first species-specific response with the second species-specific
response.
- 15 5. The method of any one of claims 1-4, wherein the screening method is
performed on a plurality of test compounds.
6. The method of claim 5, wherein the response mediated by the TLR3 signal
transduction pathway is measured quantitatively.
20
7. The method of any one of claims 1-4, wherein the functional TLR3 is expressed
in a cell.
8. The method of claim 7, wherein the cell is an isolated mammalian cell that
25 naturally expresses the functional TLR3.
9. The method of claim 7, wherein the cell is an isolated mammalian cell that does
not naturally express the functional TLR3, and wherein the cell comprises an
expression vector for TLR3.
30
10. The method of claim 9, wherein the cell is a 293 human fibroblast.

11. The method of claim 7, wherein the cell comprises an expression vector comprising an isolated nucleic acid which encodes a reporter construct selected from the group of interleukin-6-luciferase (IL-6-luc), IL-8-luc, IL-12 p40-luc, IL-12 p40- β -Gal, NF- κ B-luc, AP1-luc, IFN- α -luc, IFN- β -luc, RANTES-luc, TNF-luc, IP-
5 10-luc, I-TAC-luc, and ISRE-luc.
12. The method of claim 11, wherein the reporter construct is ISRE-luc.
13. The method of any one of claims 1-4, wherein the functional TLR3 is part of a
10 cell-free system.
14. The method of any one of claims 1-4, wherein the functional TLR3 is part of a complex with a non-TLR protein selected from the group consisting of MyD88, IL-1 receptor associated kinase 1-3 (IRAK1, IRAK2, IRAK3), tumor necrosis factor
15 receptor-associated factor 1-6 (TRAF1 - TRAF6), I κ B, NF- κ B, MyD88-adaptor-like (Mal), Toll-interleukin 1 receptor (TIR) domain-containing adapter protein (TIRAP), Tollip, Rac, and functional homologues and derivatives thereof.
15. The method of claim 14, wherein the non-TLR protein excludes MyD88.
20
16. The method of claim 2 or 3, wherein the reference immunostimulatory compound is a nucleic acid.
17. The method of claim 16, wherein the nucleic acid is a CpG nucleic acid.
25
18. The method of claim 2 or 3, wherein the reference immunostimulatory compound is a small molecule.
19. The method of any one of claims 1-4, wherein the test compound is a part of a
30 combinatorial library of compounds.

20. The method of any one of claims 1-4, wherein the test compound is a nucleic acid.
21. The method of claim 20, wherein the nucleic acid is a CpG nucleic acid.
- 5 22. The method of any one of claims 1-4, wherein the test compound is a small molecule.
- 10 23. The method of any one of claims 1-4, wherein the test compound is a polypeptide.
24. The method of any one of claims 1-4, wherein the response mediated by a TLR3 signal transduction pathway is induction of a reporter gene under control of a promoter response element selected from the group consisting of ISRE, IL-6, IL-8, 15 IL-12 p40, IFN- α , IFN- β , IFN- ω , RANTES, TNF, IP-10, and I-TAC.
25. The method of claim 24, wherein the reporter gene under control of a promoter response element is selected from the group consisting of ISRE-luc, IL-6-luc, IL-8-luc, IL-12 p40-luc, IL-12 p40- β -Gal, IFN- α -luc, IFN- β -luc, RANTES-luc, TNF-luc, 20 IP-10-luc, and I-TAC-luc.
26. The method of claim 25, wherein the reporter gene under control of a promoter response element is ISRE-luc.
- 25 27. The method of claim 24, wherein the reporter gene is selected from the group consisting of IFN- α 1-luc and IFN- α 4-luc.
28. The method of any one of claims 1-4, wherein the response mediated by a TLR3 signal transduction pathway is selected from the group consisting of (a) 30 induction of a reporter gene under control of a minimal promoter responsive to a transcription factor selected from the group consisting of AP1, NF- κ B, ATF2, IRF3, and IRF7; (b) secretion of a chemokine; and (c) secretion of a cytokine.

29. The method of claim 28, wherein the response mediated by a TLR3 signal transduction pathway is induction of a reporter gene selected from the group consisting of AP1-luc and NF- κ B-luc.
- 5
30. The method of claim 28, wherein the response mediated by a TLR3 signal transduction pathway is secretion of a type 1 IFN.
31. The method of claim 28, wherein the response mediated by a TLR3 signal transduction pathway is secretion of a chemokine selected from the group consisting of CCL5 (RANTES), CXCL9 (Mig), CXCL10 (IP-10), and CXCL11 (I-TAC).
- 10
32. The method of any one of claims 1-3, wherein the contacting a functional TLR3 with a test compound further comprises, for each test compound, contacting with the test compound at each of a plurality of concentrations.
- 15
33. The method of any one of claims 1-3, wherein the detecting is performed 6-12 hours following the contacting.
- 20
34. The method of any one of claims 1-3, wherein the detecting is performed 16-24 hours following the contacting.

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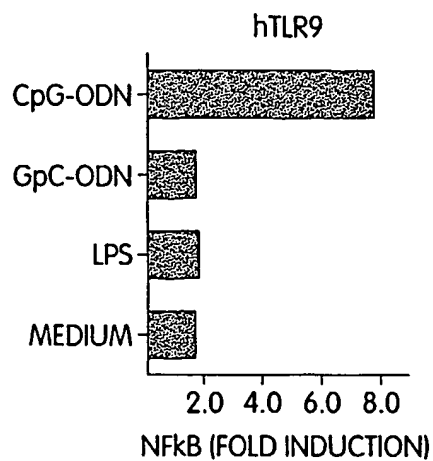


Fig. 1A

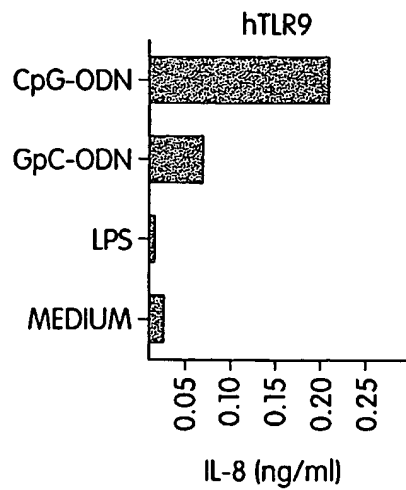


Fig. 1B

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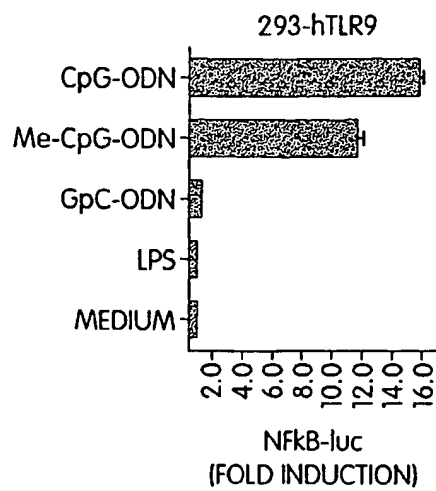


Fig. 2

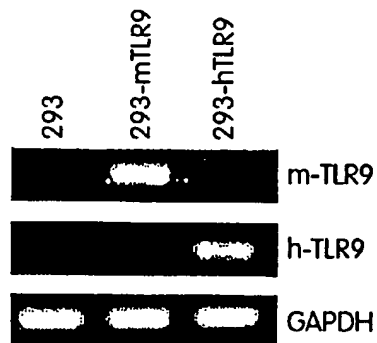


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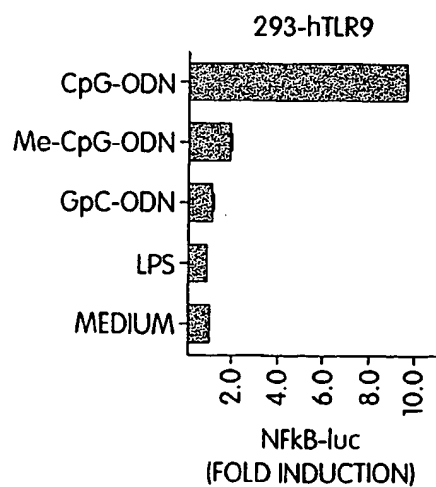


Fig. 4

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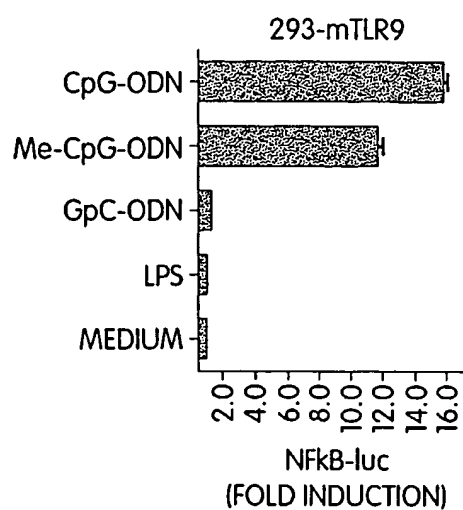


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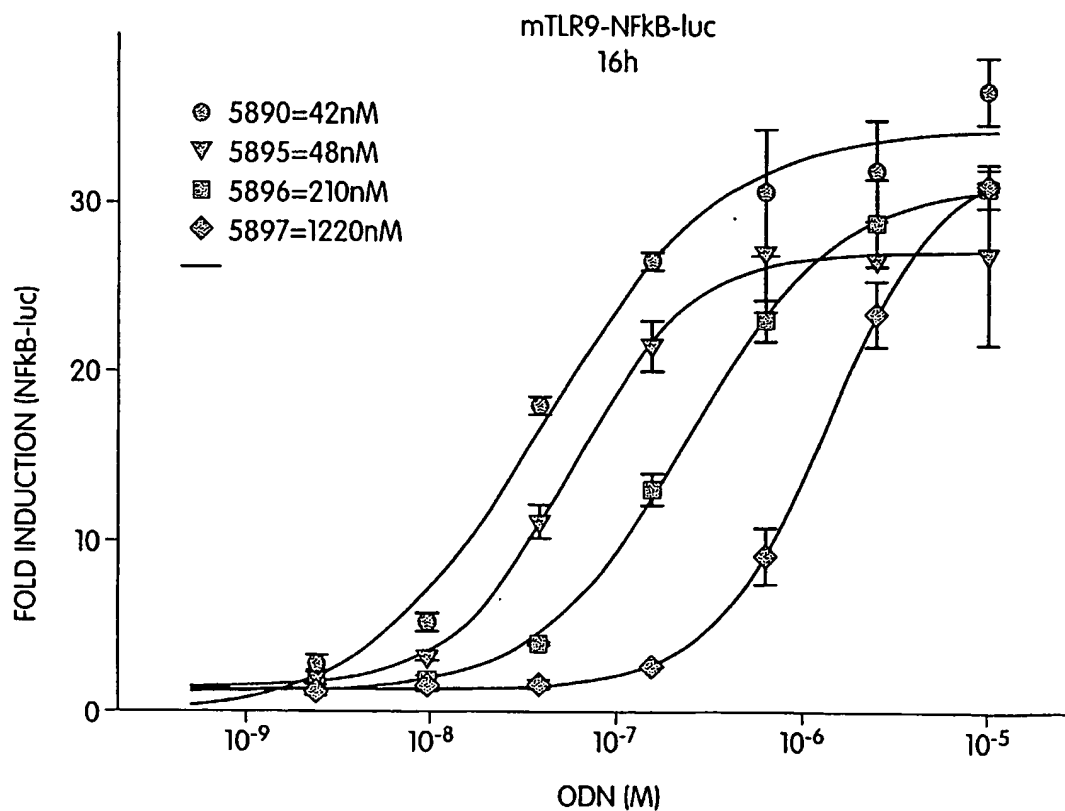


Fig. 6

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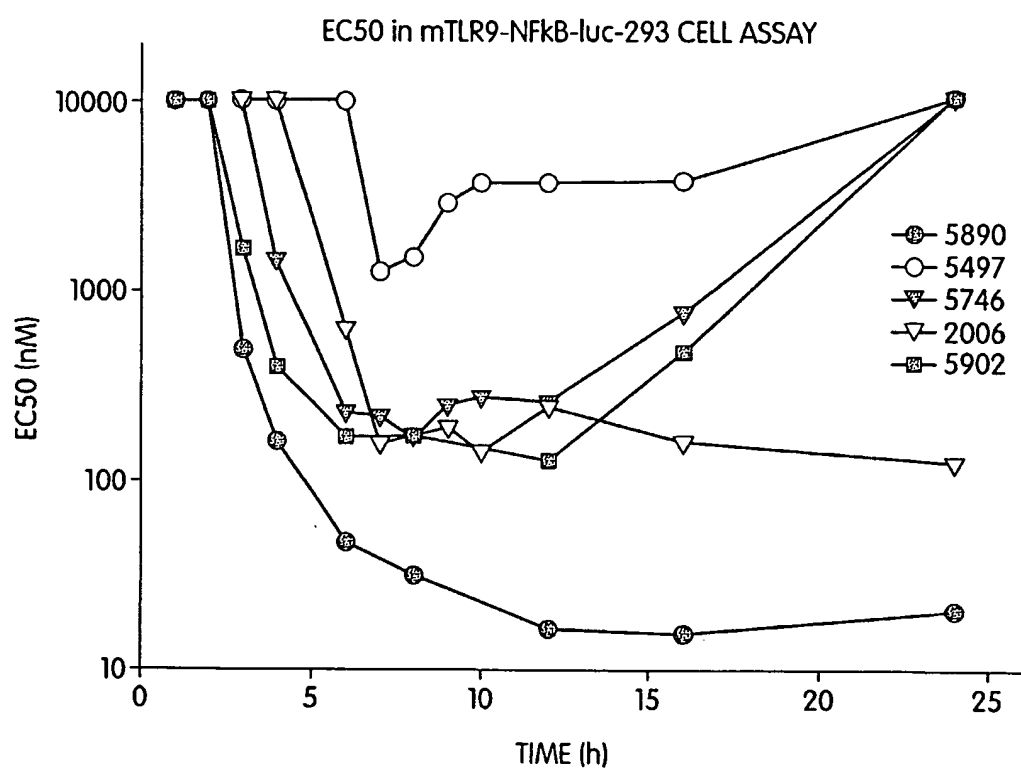


Fig. 7

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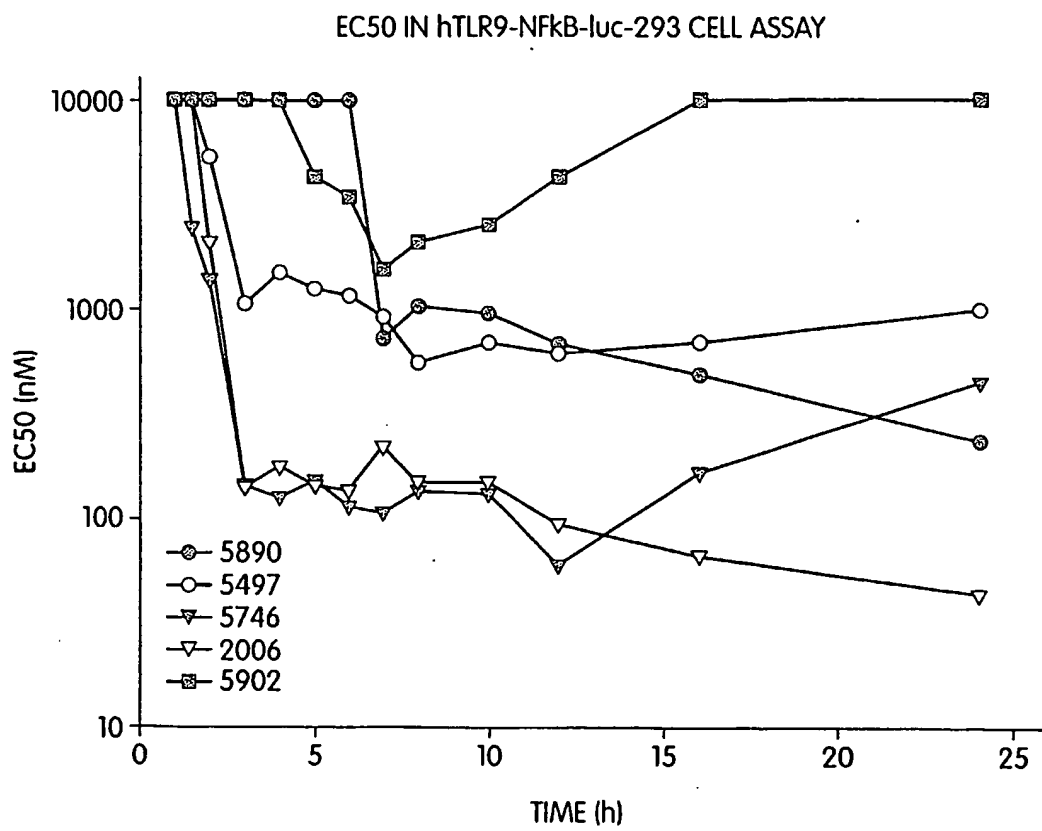


Fig. 8

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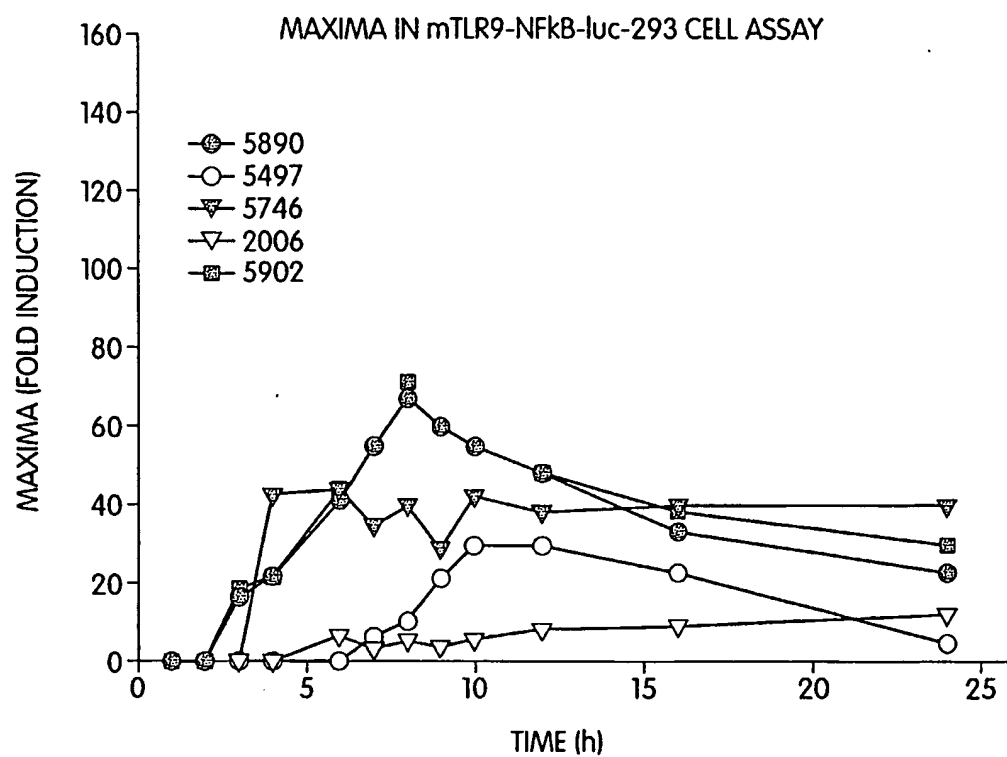


Fig. 9

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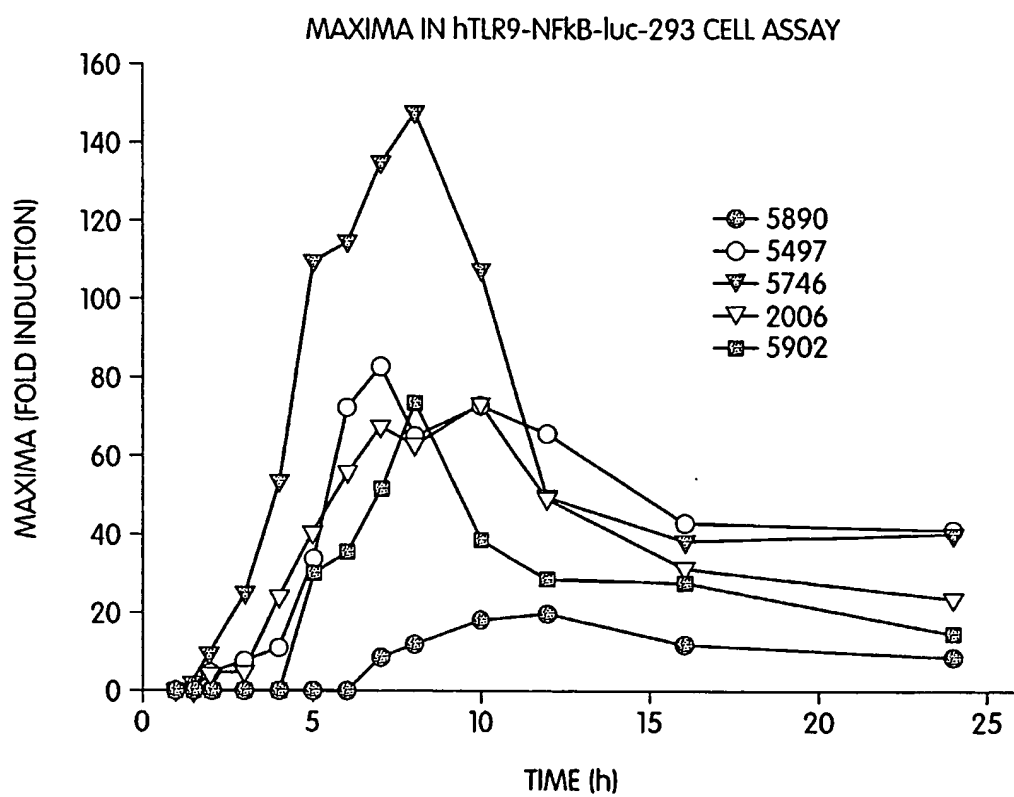


Fig. 10

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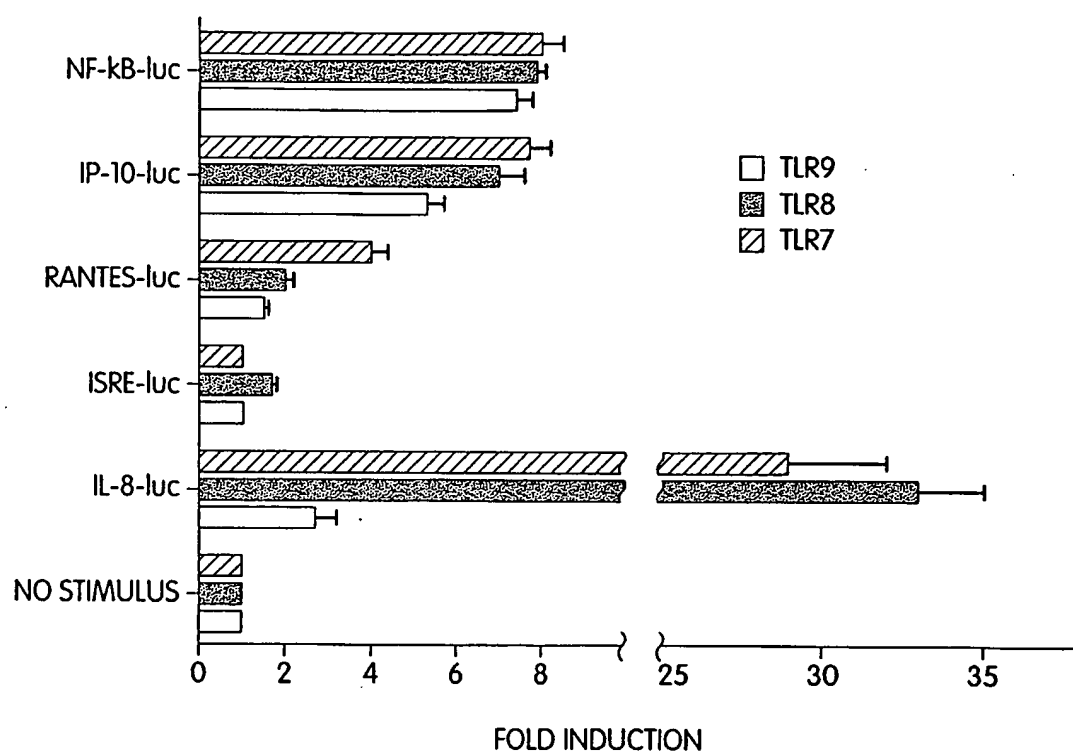


Fig. 11

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His Leu Met Ser Asn Ser Ile Gln Lys Ile Lys Asn Asn Pro Phe Val
 130 135 140

Lys Gln Lys Asn Leu Ile Thr Leu Asp Leu Ser His Asn Gly Leu Ser
 145 150 155 160

Ser Thr Lys Leu Gly Thr Gln Val Gln Leu Glu Asn Leu Gln Glu Leu
 165 170 175

Leu Leu Ser Asn Asn Lys Ile Gln Ala Leu Lys Ser Glu Glu Leu Asp
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Ile Phe Ala Asn Ser Ser Leu Lys Lys Leu Glu Leu Ser Ser Asn Gln

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Asn Ser Gln Leu Ser Thr Thr Ser Asn Thr Thr Phe Leu Gly Leu Lys 260 265 270		
Trp Thr Asn Leu Thr Met Leu Asp Leu Ser Tyr Asn Asn Leu Asn Val 275 280 285		
Val Gly Asn Asp Ser Phe Ala Trp Leu Pro Gln Leu Glu Tyr Phe Phe 290 295 300		
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Leu Phe Asn Val Arg Tyr Leu Asn Leu Lys Arg Ser Phe Thr Lys Gln 325 330 335		
Ser Ile Ser Leu Ala Ser Leu Pro Lys Ile Asp Asp Phe Ser Phe Gln 340 345 350		
Trp Leu Lys Cys Leu Glu His Leu Asn Met Glu Asp Asn Asp Ile Pro 355 360 365		
Gly Ile Lys Ser Asn Met Phe Thr Gly Leu Ile Asn Leu Lys Tyr Leu 370 375 380		
Ser Leu Ser Asn Ser Phe Thr Ser Leu Arg Thr Leu Thr Asn Glu Thr 385 390 395 400		
Phe Val Ser Leu Ala His Ser Pro Leu His Ile Leu Asn Leu Thr Lys 405 410 415		
Asn Lys Ile Ser Lys Ile Glu Ser Asp Ala Phe Ser Trp Leu Gly His 420 425 430		

Leu Glu Val Leu Asp Leu Gly Leu Asn Glu Ile Gly Gln Glu Leu Thr
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Gly Gln Glu Trp Arg Gly Leu Glu Asn Ile Phe Glu Ile Tyr Leu Ser
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Tyr Asn Lys Tyr Leu Gln Leu Thr Arg Asn Ser Phe Ala Leu Val Pro
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Ser Leu Gln Arg Leu Met Leu Arg Arg Val Ala Leu Lys Asn Val Asp
 485 490 495

Ser Ser Pro Ser Pro Phe Gln Pro Leu Arg Asn Leu Thr Ile Leu Asp
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Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn Asp Asp Met Leu Glu Gly
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Leu Glu Lys Leu Glu Ile Leu Asp Leu Gln His Asn Asn Leu Ala Arg
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Leu Trp Lys His Ala Asn Pro Gly Gly Pro Ile Tyr Phe Leu Lys Gly
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Leu Ser His Leu His Ile Leu Asn Leu Glu Ser Asn Gly Phe Asp Glu
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Ile Pro Val Glu Val Phe Lys Asp Leu Phe Glu Leu Lys Ile Ile Asp
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Leu Gly Leu Asn Asn Leu Asn Thr Leu Pro Ala Ser Val Phe Asn Asn
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Gln Val Ser Leu Lys Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr Ser
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Val Glu Lys Lys Val Phe Gly Pro Ala Phe Arg Asn Leu Thr Glu Leu
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Asp Met Arg Phe Asn Pro Phe Asp Cys Thr Cys Glu Ser Ile Ala Trp
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Phe Val Asn Trp Ile Asn Glu Thr His Thr Asn Ile Pro Glu Leu Ser
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Ser His Tyr Leu Cys Asn Thr Pro Pro His Tyr His Gly Phe Pro Val
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Arg Leu Phe Asp Thr Ser Ser Cys Lys Asp Ser Ala Pro Phe Glu Leu
690 695 700

Phe Phe Met Ile Asn Thr Ser Ile Leu Leu Ile Phe Ile Phe Ile Val
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Leu Leu Ile His Phe Glu Gly Trp Arg Ile Ser Phe Tyr Trp Asn Val
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Ser Val His Arg Val Leu Gly Phe Lys Glu Ile Asp Arg Gln Thr Glu
740 745 750

Gln Phe Glu Tyr Ala Ala Tyr Ile Ile His Ala Tyr Lys Asp Lys Asp
755 760 765

Trp Val Trp Glu His Phe Ser Ser Met Glu Lys Glu Asp Gln Ser Leu
770 775 780

Lys Phe Cys Leu Glu Glu Arg Asp Phe Glu Ala Gly Val Phe Glu Leu
785 790 795 800

Glu Ala Ile Val Asn Ser Ile Lys Arg Ser Arg Lys Ile Ile Phe Val
805 810 815

Ile Thr His His Leu Leu Lys Asp Pro Leu Cys Lys Arg Phe Lys Val
820 825 830

His His Ala Val Gln Gln Ala Ile Glu Gln Asn Leu Asp Ser Ile Ile
835 840 845

Leu Val Phe Leu Glu Glu Ile Pro Asp Tyr Lys Leu Asn His Ala Leu
850 855 860

Cys Leu Arg Arg Gly Met Phe Lys Ser His Cys Ile Leu Asn Trp Pro
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3310

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Asp Leu Pro Ser Asn Ile Thr Val Leu Asn Leu Thr His Asn Gln Leu
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Arg Arg Leu Pro Pro Thr Asn Phe Thr Arg Tyr Ser Gln Leu Ala Ile
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Leu Asp Ala Gly Phe Asn Ser Ile Ser Lys Leu Glu Pro Glu Leu Cys
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Gln Ile Leu Pro Leu Leu Lys Val Leu Asn Leu Gln His Asn Glu Leu
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Ser Gln Ile Ser Asp Gln Thr Phe Val Phe Cys Thr Asn Leu Thr Glu
 115 120 125

Leu Asp Leu Met Ser Asn Ser Ile His Lys Ile Lys Ser Asn Pro Phe
 130 135 140

Lys Asn Gln Lys Asn Leu Ile Lys Leu Asp Leu Ser His Asn Gly Leu
 145 150 155 160

Ser Ser Thr Lys Leu Gly Thr Gly Val Gln Leu Glu Asn Leu Gln Glu
 165 170 175

Leu Leu Leu Ala Lys Asn Lys Ile Leu Ala Leu Arg Ser Glu Glu Leu
 180 185 190

Glu Phe Leu Gly Asn Ser Ser Leu Arg Lys Leu Asp Leu Ser Ser Asn

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Pro Leu Lys Glu Phe Ser 210	Pro Gly Cys Phe Gln Thr 215	Ile Gly Lys Leu 220
Phe Ala Leu Leu Leu Asn 225	Asn Ala Gln Leu Asn 230	Pro His Leu Thr Glu 235 240
Lys Leu Cys Trp Glu Leu Ser 245	Asn Thr Ser Ile Gln 250	Asn Leu Ser Leu 255
Ala Asn Asn Gln Leu Leu Ala 260	Thr Ser Glu Ser Thr 265	Phe Ser Gly Leu 270
Lys Trp Thr Asn Leu Thr Gln 275	Leu Asp Leu Ser Tyr 280	Asn Asn Leu His 285
Asp Val Gly Asn Gly Ser 290	Phe Ser Tyr Leu Pro 295	Ser Leu Arg Tyr Leu 300
Ser Leu Glu Tyr Asn Asn 305	Ile Gln Arg Leu Ser 310	Pro Arg Ser Phe Tyr 315 320
Gly Leu Ser Asn Leu Arg Tyr 325	Leu Ser Leu Lys Arg 330	Ala Phe Thr Lys 335
Gln Ser Val Ser Leu Ala Ser 340	His Pro Asn Ile Asp 345	Asp Phe Ser Phe 350
Gln Trp Leu Lys Tyr Leu Glu 355	Tyr Leu Asn Met Asp 360	Asp Asn Asn Ile 365
Pro Ser Thr Lys Ser Asn Thr 370	Phe Thr Gly Leu Val 375	Ser Leu Lys Tyr 380
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Thr Phe Val Ser Leu Ala His 405	Ser Pro Leu Leu Thr 410	Leu Asn Leu Thr 415
Lys Asn His Ile Ser Lys Ile 420	Ala Asn Gly Thr Phe 425	Ser Trp Leu Gly 430

Gln Leu Arg Ile Leu Asp Leu Gly Leu Asn Glu Ile Glu Gln Lys Leu
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Ser Gly Gln Glu Trp Arg Gly Leu Arg Asn Ile Phe Glu Ile Tyr Leu
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Ser Tyr Asn Lys Tyr Leu Gln Leu Ser Thr Ser Ser Phe Ala Leu Val
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Pro Ser Leu Gln Arg Leu Met Leu Arg Arg Val Ala Leu Lys Asn Val
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Asp Ile Ser Pro Ser Pro Phe Arg Pro Leu Arg Asn Leu Thr Ile Leu
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Asp Leu Ser Asn Asn Asn Ile Ala Asn Ile Asn Glu Asp Leu Leu Glu
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Gly Leu Glu Asn Leu Glu Ile Leu Asp Phe Gln His Asn Asn Leu Ala
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Arg Leu Trp Lys Arg Ala Asn Pro Gly Gly Pro Val Asn Phe Leu Lys
 545 550 555 560

Gly Leu Ser His Leu His Ile Leu Asn Leu Glu Ser Asn Gly Leu Asp
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Glu Ile Pro Val Gly Val Phe Lys Asn Leu Phe Glu Leu Lys Ser Ile
 580 585 590

Asn Leu Gly Leu Asn Asn Leu Asn Lys Leu Glu Pro Phe Ile Phe Asp
 595 600 605

Asp Gln Thr Ser Leu Arg Ser Leu Asn Leu Gln Lys Asn Leu Ile Thr
 610 615 620

Ser Val Glu Lys Asp Val Phe Gly Pro Pro Phe Gln Asn Leu Asn Ser
 625 630 635 640

Leu Asp Met Arg Phe Asn Pro Phe Asp Cys Thr Cys Glu Ser Ile Ser
 645 650 655

Trp Phe Val Asn Trp Ile Asn Gln Thr His Thr Asn Ile Phe Glu Leu
 660 665 670

Ser Thr His Tyr Leu Cys Asn Thr Pro His His Tyr Tyr Gly Phe Pro
675 680 685

Leu Lys Leu Phe Asp Thr Ser Ser Cys Lys Asp Ser Ala Pro Phe Glu
690 695 700

Leu Leu Phe Ile Ile Ser Thr Ser Met Leu Leu Val Phe Ile Leu Val
705 710 715 720

Val Leu Leu Ile His Ile Glu Gly Trp Arg Ile Ser Phe Tyr Trp Asn
725 730 735

Val Ser Val His Arg Ile Leu Gly Phe Lys Glu Ile Asp Thr Gln Ala
740 745 750

Glu Gln Phe Glu Tyr Thr Ala Tyr Ile Ile His Ala His Lys Asp Arg
755 760 765

Asp Trp Val Trp Glu His Phe Ser Pro Met Glu Glu Gln Asp Gln Ser
770 775 780

Leu Lys Phe Cys Leu Glu Glu Arg Asp Phe Glu Ala Gly Val Leu Gly
785 790 795 800

Leu Glu Ala Ile Val Asn Ser Ile Lys Arg Ser Arg Lys Ile Ile Phe
805 810 815

Val Ile Thr His His Leu Leu Lys Asp Pro Leu Cys Arg Arg Phe Lys
820 825 830

Val His His Ala Val Gln Gln Ala Ile Glu Gln Asn Leu Asp Ser Ile
835 840 845

Ile Leu Ile Phe Leu Gln Asn Ile Pro Asp Tyr Lys Leu Asn His Ala
850 855 860

Leu Cys Leu Arg Arg Gly Met Phe Lys Ser His Cys Ile Leu Asn Trp
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Ala Leu Gly Ser Arg Asn Ser Ala His
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<213> Homo sapiens

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Leu Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu
35 40 45

Phe Leu Lys Ser Val Pro His Phe Ser Met Ala Ala Pro Arg Gly Asn
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Val Thr Ser Leu Ser Leu Ser Ser Asn Arg Ile His His Leu His Asp
65 70 75 80

Ser Asp Phe Ala His Leu Pro Ser Leu Arg His Leu Asn Leu Lys Trp
85 90 95

Asn Cys Pro Pro Val Gly Leu Ser Pro Met His Phe Pro Cys His Met
100 105 110

Thr Ile Glu Pro Ser Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
115 120 125

Asn Leu Ser Tyr Asn Asn Ile Met Thr Val Pro Ala Leu Pro Lys Ser
130 135 140

Leu Ile Ser Leu Ser Leu Ser His Thr Asn Ile Leu Met Leu Asp Ser
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Ala Ser Leu Ala Gly Leu His Ala Leu Arg Phe Leu Phe Met Asp Gly
165 170 175

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Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr

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Arg Cys Asp His Ala Pro Asn Pro Cys Met Glu Cys Pro Arg His Phe		
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Pro Gln Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly		
275	280	285
Leu Val Leu Lys Asp Ser Ser Leu Ser Trp Leu Asn Ala Ser Trp Phe		
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Arg Gly Leu Gly Asn Leu Arg Val Leu Asp Leu Ser Glu Asn Phe Leu		
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Tyr Lys Cys Ile Thr Lys Thr Lys Ala Phe Gln Gly Leu Thr Gln Leu		
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Arg Lys Leu Asn Leu Ser Phe Asn Tyr Gln Lys Arg Val Ser Phe Ala		
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His Leu Ser Leu Ala Pro Ser Phe Gly Ser Leu Val Ala Leu Lys Glu		
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Leu Asp Met His Gly Ile Phe Phe Arg Ser Leu Asp Glu Thr Thr Leu		
370	375	380
Arg Pro Leu Ala Arg Leu Pro Met Leu Gln Thr Leu Arg Leu Gln Met		
385	390	395 400
Asn Phe Ile Asn Gln Ala Gln Leu Gly Ile Phe Arg Ala Phe Pro Gly		
405	410	415
Leu Arg Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ser Glu		
420	425	430

Leu Thr Ala Thr Met Gly Glu Ala Asp Gly Gly Glu Lys Val Trp Leu
 435 440 445

Gln Pro Gly Asp Leu Ala Pro Ala Pro Val Asp Thr Pro Ser Ser Glu
 450 455 460

Asp Phe Arg Pro Asn Cys Ser Thr Leu Asn Phe Thr Leu Asp Leu Ser
 465 470 475 480

Arg Asn Asn Leu Val Thr Val Gln Pro Glu Met Phe Ala Gln Leu Ser
 485 490 495

His Leu Gln Cys Leu Arg Leu Ser His Asn Cys Ile Ser Gln Ala Val
 500 505 510

Asn Gly Ser Gln Phe Leu Pro Leu Thr Gly Leu Gln Val Leu Asp Leu
 515 520 525

Ser Arg Asn Lys Leu Asp Leu Tyr His Glu His Ser Phe Thr Glu Leu
 530 535 540

Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Gly
 545 550 555 560

Met Gln Gly Val Gly His Asn Phe Ser Phe Val Ala His Leu Arg Thr
 565 570 575

Leu Arg His Leu Ser Leu Ala His Asn Asn Ile His Ser Gln Val Ser
 580 585 590

Gln Gln Leu Cys Ser Thr Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn
 595 600 605

Ala Leu Gly His Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe
 610 615 620

Gln Gly Leu Ser Gly Leu Ile Trp Leu Asp Leu Ser Gln Asn Arg Leu
 625 630 635 640

His Thr Leu Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln
 645 650 655

Val Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser
 660 665 670

Leu His Phe Leu Pro Lys Leu Glu Val Leu Asp Leu Ala Gly Asn Arg
 675 680 685

Leu Lys Ala Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg
 690 695 700

Arg Leu Asp Val Ser Cys Asn Ser Ile Ser Phe Val Ala Pro Gly Phe
 705 710 715 720

Phe Ser Lys Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala
 725 730 735

Leu Lys Thr Val Asp His Ser Trp Phe Gly Pro Leu Ala Ser Ala Leu
 740 745 750

Gln Ile Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala
 755 760 765

Ala Phe Met Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu
 770 775 780

Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Leu Ser
 785 790 795 800

Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Ala Leu Ser Trp
 805 810 815

Asp Cys Phe Ala Leu Ser Leu Leu Ala Val Ala Leu Gly Leu Gly Val
 820 825 830

Pro Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His
 835 840 845

Leu Cys Leu Ala Trp Leu Pro Trp Arg Gly Arg Gln Ser Gly Arg Asp
 850 855 860

Glu Asp Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Thr Gln
 865 870 875 880

Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Gly Gln Leu Glu
 885 890 895

Glu Cys Arg Gly Arg Trp Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp
 900 905 910

Trp Leu Pro Gly Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr
 915 920 925

Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser
 930 935 940

Gly Leu Leu Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
 945 950 955 960

Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Ser Pro Asp Gly Arg
 965 970 975

Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
 980 985 990

Leu Leu Trp Pro His Gln Pro Ser Gly Gln Arg Ser Phe Trp Ala Gln
 995 1000 1005

Leu Gly Met Ala Leu Thr Arg Asp Asn His His Phe Tyr Asn Arg
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Asn Phe Cys Gln Gly Pro Thr Ala Glu
 1025 1030

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 <211> 3200
 <212> DNA
 <213> Mus musculus

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 ctgttctga agtctgtacc ccgtttctct gcggcagcat cctgctccaa catcaccgcg 240
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 ctgaacctga gctataatgg tatcaccact gtgccccgac tgcccagctc cctggtgaat 480
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<210> 8

<211> 1032

<212> PRT

<213> Mus musculus

<400> 8

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Leu Pro Cys Glu Leu Lys Pro His Gly Leu Val Asp Cys Asn Trp Leu
 35 40 45

Phe Leu Lys Ser Val Pro Arg Phe Ser Ala Ala Ala Ser Cys Ser Asn
 50 55 60

Ile Thr Arg Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asn
 65 70 75 80

Ser Asp Phe Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp

	85		90		95
Asn Cys Pro	Pro Thr Gly Leu Ser	Pro Leu His Phe Ser	Cys His Met		
	100	105	110		
Thr Ile Glu	Pro Arg Thr Phe Leu Ala Met Arg Thr	Leu Glu Glu Leu			
	115	120	125		
Asn Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Arg	Leu Pro Ser Ser				
	130	135	140		
Leu Val Asn Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala					
	145	150	155		160
Asn Ser Leu Ala Gly Leu Tyr Ser Leu Arg Val Leu Phe Met Asp Gly					
	165	170	175		
Asn Cys Tyr Tyr Lys Asn Pro Cys Thr Gly Ala Val Lys Val Thr Pro					
	180	185	190		
Gly Ala Leu Leu Gly Leu Ser Asn Leu Thr His Leu Ser Leu Lys Tyr					
	195	200	205		
Asn Asn Leu Thr Lys Val Pro Arg Gln Leu Pro Pro Ser Leu Glu Tyr					
	210	215	220		
Leu Leu Val Ser Tyr Asn Leu Ile Val Lys Leu Gly Pro Glu Asp Leu					
	225	230	235		240
Ala Asn Leu Thr Ser Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg					
	245	250	255		
Arg Cys Asp His Ala Pro Asn Pro Cys Ile Glu Cys Gly Gln Lys Ser					
	260	265	270		
Leu His Leu His Pro Glu Thr Phe His His Leu Ser His Leu Glu Gly					
	275	280	285		
Leu Val Leu Lys Asp Ser Ser Leu His Thr Leu Asn Ser Ser Trp Phe					
	290	295	300		
Gln Gly Leu Val Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu					
	305	310	315		320

Tyr Glu Ser Ile Asn His Thr Asn Ala Phe Gln Asn Leu Thr Arg Leu
 325 330 335

Arg Lys Leu Asn Leu Ser Phe Asn Tyr Arg Lys Lys Val Ser Phe Ala
 340 345 350

Arg Leu His Leu Ala Ser Ser Phe Lys Asn Leu Val Ser Leu Gln Glu
 355 360 365

Leu Asn Met Asn Gly Ile Phe Phe Arg Ser Leu Asn Lys Tyr Thr Leu
 370 375 380

Arg Trp Leu Ala Asp Leu Pro Lys Leu His Thr Leu His Leu Gln Met
 385 390 395 400

Asn Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Thr Phe Arg Ala
 405 410 415

Leu Arg Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Pro Ser Thr
 420 425 430

Leu Ser Glu Ala Thr Pro Glu Glu Ala Asp Asp Ala Glu Gln Glu Glu
 435 440 445

Leu Leu Ser Ala Asp Pro His Pro Ala Pro Leu Ser Thr Pro Ala Ser
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Lys Asn Phe Met Asp Arg Cys Lys Asn Phe Lys Phe Thr Met Asp Leu
 465 470 475 480

Ser Arg Asn Asn Leu Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu
 485 490 495

Ser Arg Leu Gln Cys Leu Ser Leu Ser His Asn Ser Ile Ala Gln Ala
 500 505 510

Val Asn Gly Ser Gln Phe Leu Pro Leu Thr Asn Leu Gln Val Leu Asp
 515 520 525

Leu Ser His Asn Lys Leu Asp Leu Tyr His Trp Lys Ser Phe Ser Glu
 530 535 540

Leu Pro Gln Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe
 545 550 555 560

Ser Met Lys Gly Ile Gly His Asn Phe Ser Phe Val Ala His Leu Ser
 565 570 575

Met Leu His Ser Leu Ser Leu Ala His Asn Asp Ile His Thr Arg Val
 580 585 590

Ser Ser His Leu Asn Ser Asn Ser Val Arg Phe Leu Asp Phe Ser Gly
 595 600 605

Asn Gly Met Gly Arg Met Trp Asp Glu Gly Gly Leu Tyr Leu His Phe
 610 615 620

Phe Gln Gly Leu Ser Gly Leu Leu Lys Leu Asp Leu Ser Gln Asn Asn
 625 630 635 640

Leu His Ile Leu Arg Pro Gln Asn Leu Asp Asn Leu Pro Lys Ser Leu
 645 650 655

Lys Leu Leu Ser Leu Arg Asp Asn Tyr Leu Ser Phe Phe Asn Trp Thr
 660 665 670

Ser Leu Ser Phe Leu Pro Asn Leu Glu Val Leu Asp Leu Ala Gly Asn
 675 680 685

Gln Leu Lys Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu
 690 695 700

Gln Lys Leu Asp Val Ser Ser Asn Ser Ile Val Ser Val Val Pro Ala
 705 710 715 720

Phe Phe Ala Leu Ala Val Glu Leu Lys Glu Val Asn Leu Ser His Asn
 725 730 735

Ile Leu Lys Thr Val Asp Arg Ser Trp Phe Gly Pro Ile Val Met Asn
 740 745 750

Leu Thr Val Leu Asp Val Arg Ser Asn Pro Leu His Cys Ala Cys Gly
 755 760 765

Ala Ala Phe Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly
 770 775 780

Leu Ala Asn Gly Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg
 785 790 795 800

Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Val Leu Ser
805 810 815

Trp Asp Cys Phe Gly Leu Ser Leu Leu Ala Val Ala Val Gly Met Val
820 825 830

Val Pro Ile Leu His His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe
835 840 845

His Leu Cys Leu Ala Trp Leu Pro Leu Leu Ala Arg Ser Arg Arg Ser
850 855 860

Ala Gln Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln
865 870 875 880

Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu
885 890 895

Glu Arg Arg Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Asp Arg Asp
900 905 910

Trp Leu Pro Gly Gln Thr Leu Phe Glu Asn Leu Trp Ala Ser Ile Tyr
915 920 925

Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser
930 935 940

Gly Leu Leu Arg Thr Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
945 950 955 960

Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala His
965 970 975

Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
980 985 990

Leu Phe Trp Pro Gln Gln Pro Asn Gly Gln Gly Gly Phe Trp Ala Gln
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Leu Ser Thr Ala Leu Thr Arg Asp Asn Arg His Phe Tyr Asn Gln
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Asn Phe Cys Arg Gly Pro Thr Ala Glu

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1030

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<220>
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<210> 10
 <211> 37
 <212> DNA
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<220>
 <223> Synthetic oligonucleotide

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 gaaagaattc ttaatgtaca gagtttttgg atccaag 37

<210> 11
 <211> 670
 <212> DNA
 <213> Homo sapiens

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 cagctacaaa 670

<210> 12

<211> 300
 <212> DNA
 <213> Homo sapiens

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 <211> 1031
 <212> DNA
 <213> Homo sapiens

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 <213> Homo sapiens

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<220>
 <223> Synthetic oligonucleotide

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<210> 16
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<220>
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<400> 16
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<210> 17
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 <222> (2)..(2)
 <223> n = m5c

<220>
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<223> n = m5c

<220>
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<220>
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24

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<220>
<223> Synthetic oligonucleotide

<400> 18
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20

<210> 19
<211> 20
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<220>
<223> Synthetic oligonucleotide

<400> 19
tccatgagct tcctgatgct

20

<210> 20
<211> 20
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<213> Artificial sequence

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<222> (8)..(8)
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<210> 21
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<220>
<223> Synthetic oligonucleotide

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<210> 22
<211> 30
<212> DNA
<213> Artificial sequence

<220>
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<400> 22
caaagagggt ttgccatgc agccagtcgc

30

<210> 23
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 23
cgagattggc tgcattggcca gacgctcttc

30

<210> 24
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Synthetic oligonucleotide

<400> 24
gaagagcgtc tggccatgca gccaatctcg

30

<210> 25
<211> 15
<212> DNA
<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 25

ggcctcagca tcttt

15

<210> 26

<211> 15

<212> DNA

<213> Artificial sequence

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<223> Synthetic oligonucleotide

<400> 26

ggcctatcga ttttt

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<210> 27

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 27

gggttcccag tgaga

15

<210> 28

<211> 15

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 28

gggttatcga ttaga

15

<210> 29

<211> 34

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 29

cagctccagg gcctatcgat tttgcacag gacc

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